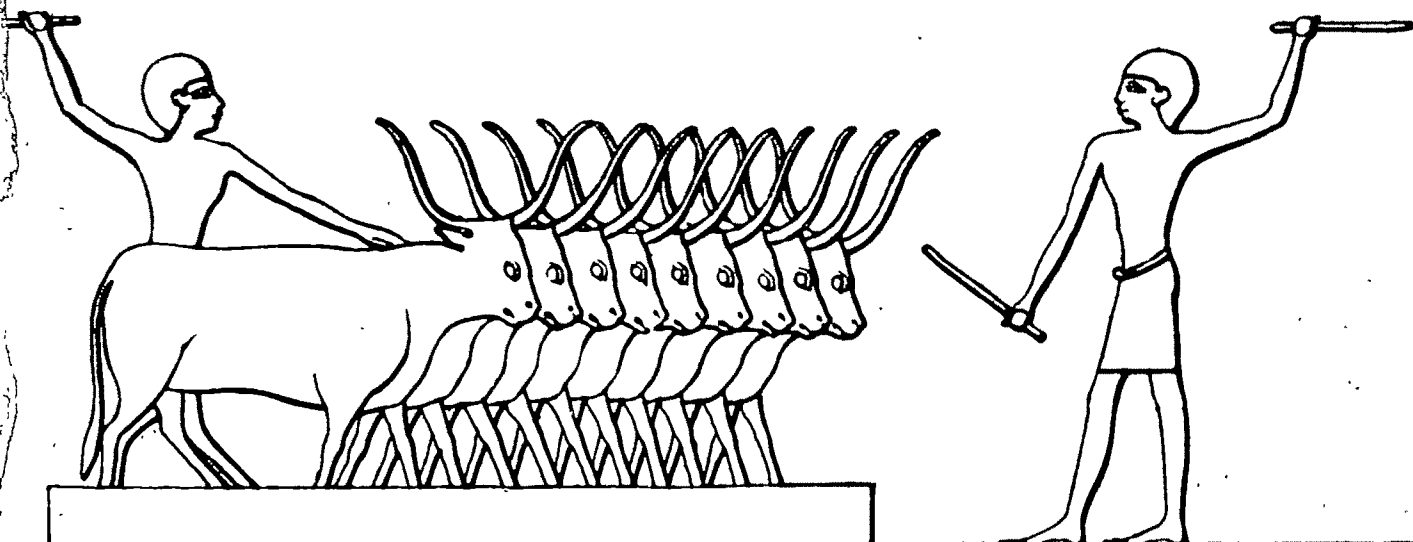
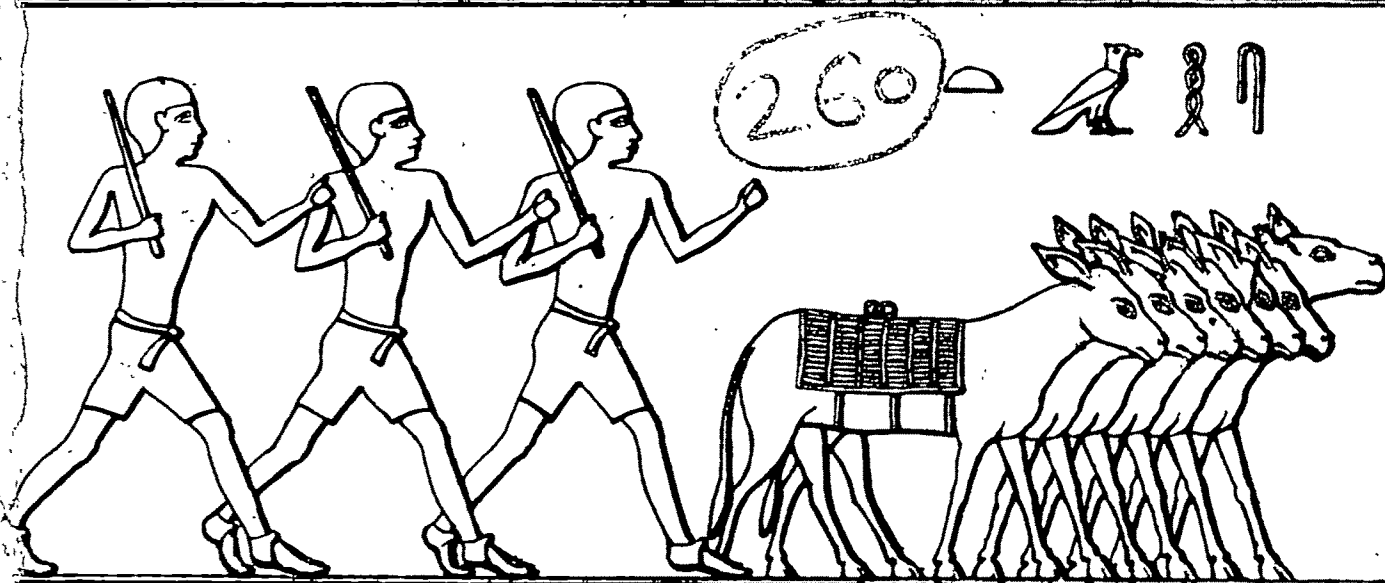
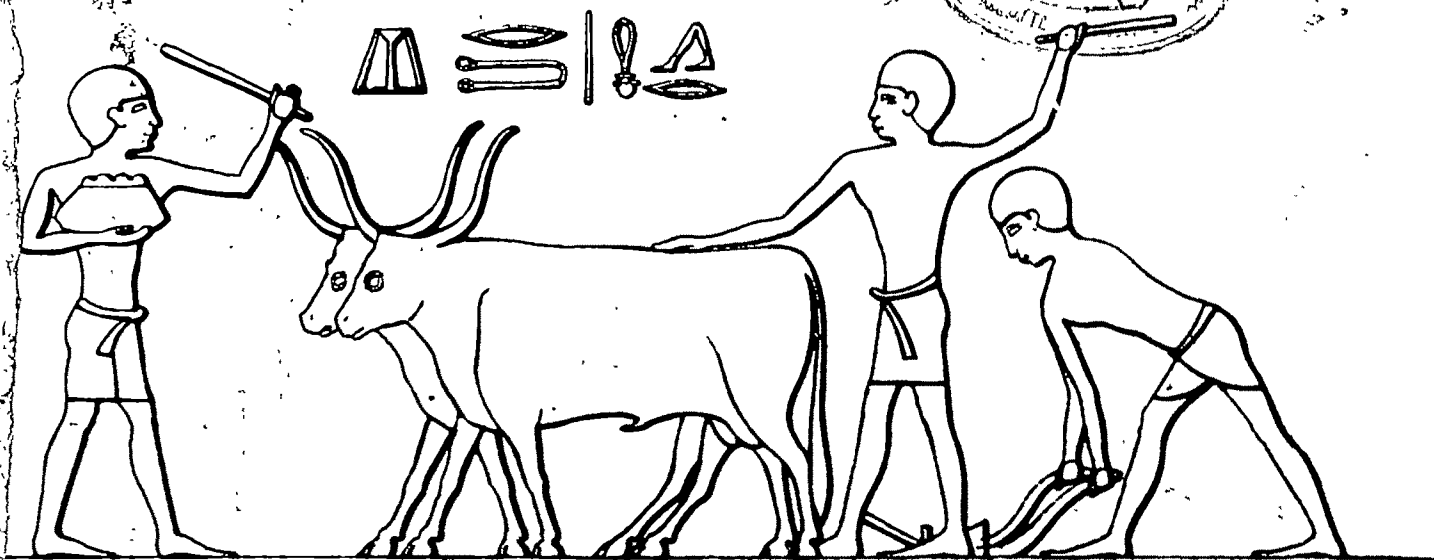


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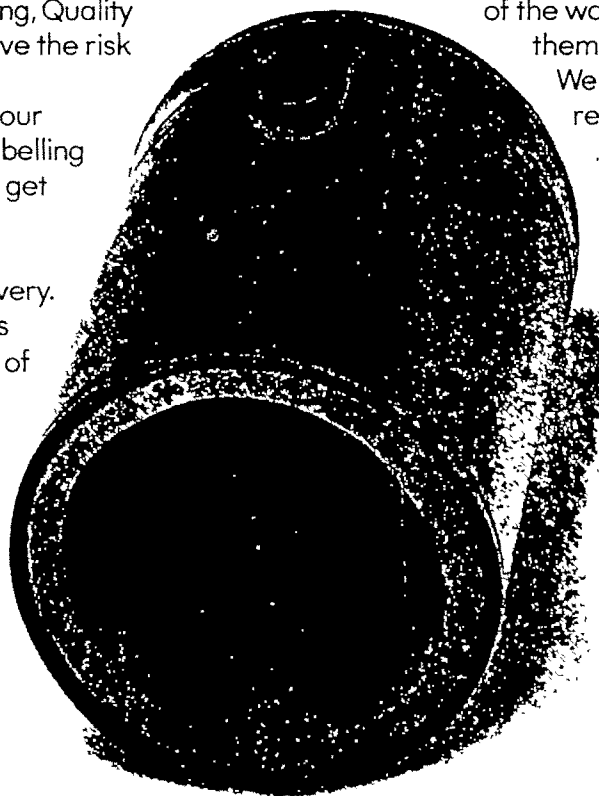
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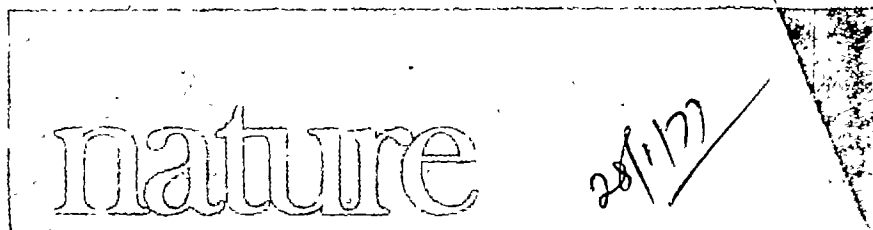
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Egyptian agricultural scene from
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(Mansell Collection)

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Ideology and the Natural Sciences.
(Michael Freeman)

J. R. Ravetz reviews two books edited
by Hilary and Steven Rose in the
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WE published a brief statement of *Nature's* editorial procedures on page 1 of the issue of November 6, 1975 in the hope that it would help readers and authors to understand how we reach decisions. We also promised to update the statement annually. A year later we have to report that our editorial practice is not substantially different. We would however like to use the opportunity to take up some general points concerning the style of *Nature* papers.

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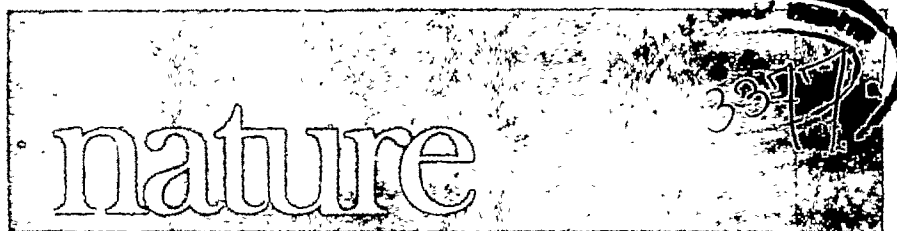
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Microtubules polymerised *in vitro* and
stained with the PAP-procedure
(Magnification $\times 100,000$)
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British Antarctic Survey base at King
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Caspar, one of the Three Kings, and
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[Sonia Halliday]

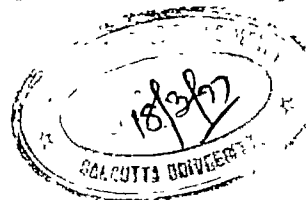
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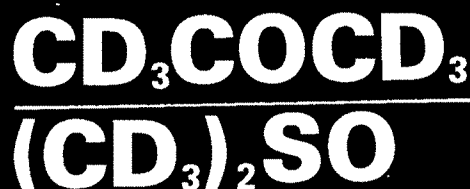
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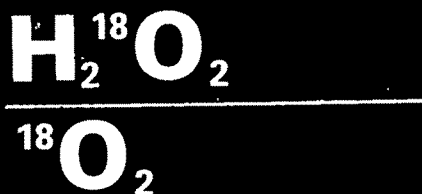
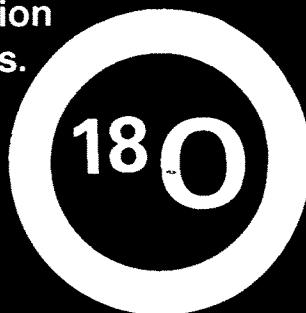
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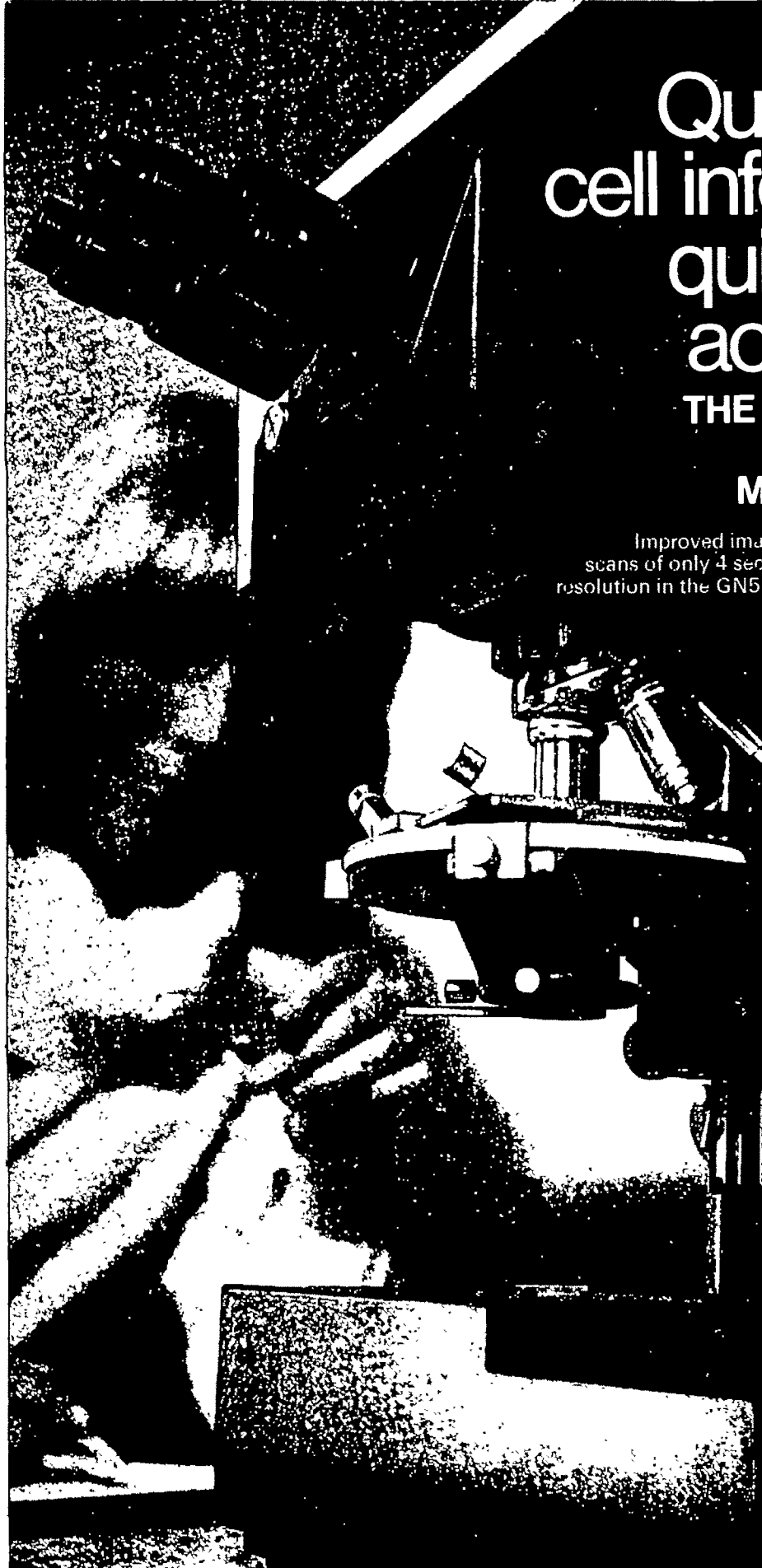
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Erratum—just before going to press we noticed that on page 529, in the list of authors, the name of D. C. Soreide had erroneously been included twice. The last author's name in the list of contributors from the Clarendon Laboratory, Oxford, should read D. N. Stacey.

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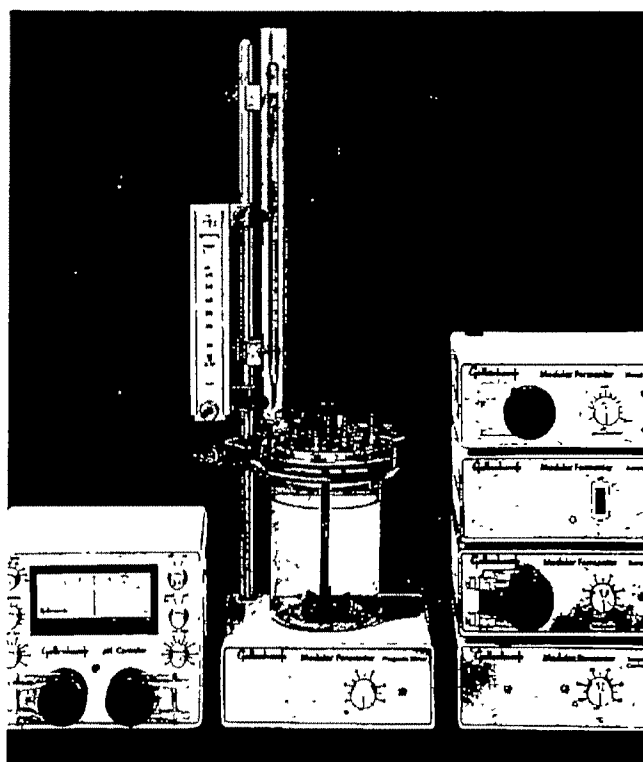
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A 'family' of genetically identical
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 white spot on the back of each animal
 is a piece of skin grafted from the
 belly of another animal in the group.
 See page 642.

Photo: M. Pazdera; Composition: H. Stahlberger
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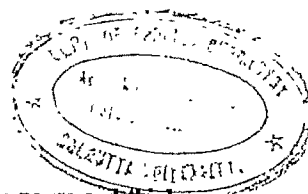
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● Review articles should be aimed at a relatively wide readership. Many reviews are invited, but submitted articles may also be accepted; it is advisable to consult us before writing a review article.

● Articles may be up to 3,000 words long with at most six displayed items (figures and tables); they are reports of major research developments.

● Letters are brief reports of original research of unusual and wide interest, not in general longer than 1,000 words; they have at most three or four displayed items.

● 'Matters Arising' permits short discussion (up to 300 words) of papers that have recently appeared in *Nature*.

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Typing (including references) should be double spaced. The title should be brief and informative. Pages should be numbered. References, tables and figure legends should start on separate pages. Experimental detail vital to the paper yet which would interrupt the narrative is best placed in the figure legends. Units should conform to the *Système International*. Greek characters should be identified in the margin on their first appearance. Equations should occupy single lines if possible; $\exp(a)$ is preferred to e^a if ' a ' is more than one character.

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This issue of *Nature* is a double issue dated December 23/30. The next issue will be published on January 6, 1977.



There will be two book supplements in the Spring of 1977. Our usual Spring Book Supplement will be published on April 28, and on March 3 we will be publishing an extensive review of recent student books.



We shall continue throughout 1977 to publish a bi-monthly author index in addition to our more comprehensive annual index. The author index is generally included in the first issue of a new volume.



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**M MACMILLAN
PRESS**

nature

November 4, 1976

Is it taking a liberty?

THE number of lectures and conferences devoted to it is growing all the time. Barely a day passes when there isn't some discussion of it on radio or television or in the newspapers. The subject is the "nuclear issue", and the immediate reason is the decision, due now but delayed a few months, on whether to go ahead in Britain with a demonstration commercial fast breeder reactor. The Minister responsible, Mr Anthony Wedgwood Benn, called for a public debate, and he's getting it. That there might be no "answer" seems to be distracting no one from the search.

The latest contribution comes in a joint publication from Friends of the Earth, the Council for the Protection of Rural England and the National Council of Civil Liberties called *Nuclear Prospects*. Subtitled "A Comment on the Individual, the State and Nuclear Power", it begins to fill a widening hole in the debate, namely a consideration of the social and political implications of nuclear power. The two authors have written what they call a speculative and highly conjectural paper explicitly with the fast breeder decision in mind.

The authors say a postulated commitment to the fast breeder, involving hundreds of tonnes of plutonium-enriched fuel, thousands of fuel shipments annually and some 100 reactors (50 of them breeders), would pose security problems even more enormous than those already caused by existing threats of theft and sabotage. Any attempt to overcome them would extend vastly the existing system of surveillance, policing and vetting inside the industry, and would provide justification for their further extension outside too. Civil liberties would be so threatened that it might be easier for even the Minister himself not to be answerable to the public.

Equally, their argument goes, electricity authorities wanting sites for nuclear stations could clash with local people who, demanding a say in what happens to land, attract wider anti-nuclear sentiment. That would threaten civil discord through direct action if people were unconvinced of the efficacy of parliamentary scrutiny and suspicious of the independence of the Nuclear Installations Inspectorate. Altogether the possible consequences make nuclear power an issue not to be argued simply at a technical level.

Certain obvious points are to be made in confronting these arguments. The security problems posed now by theft, terrorism, sabotage and bombs—even by existing stocks of plutonium—already raise serious questions about civil liberties. Countries cannot opt out of the risks unilaterally if neighbouring countries are "going plutonium". And the social consequences of an insufficiency of energy could be as dire as any caused through the use of fast breeders.

Nuclear Prospects acknowledges the first two of these points, quickly (perhaps too quickly) disposing of them. The authors wonder if civil society would adapt to standards of military security for the benefits of plutonium-based power; perhaps it might. They contend that there is "a world of difference" between exceptional precautions to combat terrorism and the steady erosion of rights threatened by the day-to-day use of plutonium; perhaps there is. But they barely address themselves to the third point, the very one that makes the first two interesting at all.

This may be because there isn't much discussion generally of the matter. Certainly if there is now any received truth in the "great debate" it is the premise that the need for energy will increase and go on increasing in a way that makes the fast breeder decision a decision that must be taken and taken soon. Voluminous documentary support exists for that view, and it cuts little ice at the moment to say that since 1971 no generating plant has been ordered (the companies concerned are reeling) because of simultaneous and seemingly unending inflation and recession.

But consider. Britain, some say, is suffering a long term decline to a subservient neocolonial status in Europe and the West; if so, her predicted energy problems (though not perhaps the world's) could arise even further into the future than presently projected. That is more grist to the mill of those already looking to a comparably financed effort in energy conservation and such alternatives as wave and solar energy to tide them over to a day when fusion or hydrogen might offer inexhaustible supplies of energy. These people imagine a nuclear interlude for Britain, not a nuclear future. Is this really a fantasy? The matter needs more discussion, not just by scientists; it cannot be divorced from a discussion of Britain's future when the cost is so gargantuan.

For consider again. Even those who acknowledge, in strictly energy terms and not with an eye to Britain's electricity industry, the need for a fast breeder, recognise that because of the resources involved the project could only succeed through international collaboration. For Britain, that means with either the French and Germans or with the Americans. The question of who will have her, assuming she can even afford to buy in, is not being discussed in public. But it makes a big difference to the value of any mere internal debate.

Perhaps it is unfair to criticise *Nuclear Prospects* on points that are beyond its intended brief; it certainly deserves praise for trying to fill a great gap in the debate. Mr Benn's decision is mostly symbolic. The debate is far from ritualistic. □

An open letter to the Health and Safety Executive

Michael Ashburner of the Department of Genetics at the University of Cambridge gives his view on the UK Health and Safety Executive's recent proposals for regulating genetic manipulation experiments

Dear Sirs,

The Health and Safety Executive is, I hope, engaging in that popular sport, kite flying. Worried, perhaps, by the mutant monsters so vividly portrayed on television every Saturday evening in *Dr Who*, you write, in your recent Consultative Document "Compulsory Notification of Proposed Genetic Experiments in the Manipulation of Microorganisms":

No person shall carry on any activity intended to alter or likely to alter the genetic constitution of *any microorganism* unless he has given to the Health and Safety Executive notice, in a form approved by the Executive for the purposes of these Regulations, of his *intention* to carry on that activity (Draft Regulation 2 of the Health and Safety (Genetic Manipulations) Regulations 1976). (my italics).

Two recent UK Government reports, those of the Working Parties chaired by Lord Ashby (Cmnd 5880; January 1975) and Professor Sir Robert Williams (Cmnd 6600; August 1976), have considered the problems posed by the recent advances in molecular biology that make it possible to introduce, in a form in which it may replicate and perhaps be transcribed and translated, DNA from "foreign" organisms (including man) into microbial hosts. Both reports considered that such experiments may lead to great social benefit but that they may be dangerous. In view of the potential dangers of some experiments of this type the Williams' Report drafted a Code of Practice to be adhered to when doing such work. This Code lays down "containment" procedures of varying levels of severity to be used for different types of experiment. In effect this will mean that because of the cost of such facilities, these experiments will be done in relatively few laboratories under the strictest supervision.

As you know, the scientific community (a pompous but useful term) is deeply divided concerning the problems that might arise from the use of these new techniques. However, the

"moderate" view is probably that the recommendations of the Williams Report (and their equivalents in the USA) are reasonable and, until we know more about the reality of the dangers, will have to be lived with. Your draft regulation is, I understand, an attempt to give the Code of Practice legal teeth and to place its administration in the hands of the Health and Safety Executive.

The Advisory Group recommended by Williams would play a subservient role to the Health and Safety Executive, despite the fact that this Group alone would include not only technically competent scientists but also "individuals able to take account of the interests of employees and the general public". A central feature of the Advisory Group is that it would "command the respect of the public as well as of the scientific community". If the advice that such a group gives can be overridden by you it would certainly lose this respect and be weakened as a result. The need for the Advisory Group is not simply, as you imply, so that the scientists would not suffer from "unnecessary delay". It is envisaged as taking on a far more important role, independently referring experimental protocols and serving as a channel by means of which new safety features can quickly reach those most directly concerned, in the laboratory.

Of course a weakness of the Williams report is that scant attention is paid to the problems that arise if such techniques come to be widespread in industry. It would make little sense if academic scientists submitted to an essentially voluntary control and industrial scientists were either not controlled or had to submit to a legal control. Accepting that some form of control is required and that this control applies equally to industry, universities and so on—a by no means universal view—the question is, how best should it be applied? Your answers to this question are your draft regulations. I

wish to point out that these regulations, especially regulation 2, are bad from three points of view.

- You remove the Advisory Group or any similar body from any central role in the dialogue that must exist between those who do the experiments and those who administer the laws under which they are done. With respect, sir, you do not have the standing in the scientific community required for this job.

- Instead of being content with covering just the "genetic engineering" experiments your draft regulation would control the *whole of microbial genetics*. You do this despite the lack of any evidence that such control is required to protect public health and safety. The consequences of wording the regulation in this way are very serious. It would make innovation in this important field of study very difficult indeed. Furthermore, the burden on a scientist communicating with you in advance the protocol of his every experiment, for no obvious reason, would be so great that you would lose the confidence and goodwill of the scientific community. If this were to happen the dangers might be very real since you rely upon this community to draw both real and potential hazards to your attention. Remember that it was the scientists who made possible the "genetic engineering" experiments who brought their concern to public attention (*Nature* 250, 175). If a result of the public debate so actively encouraged by them is that scientists find their day to day activities encumbered with endless red tape, some may be reluctant to speak out quite so publicly in the future—and that could lead to a disaster that any subsequent legal process could do little to remedy. It would be a tragedy if certain types of scientific work were driven "underground" as a consequence of a ridiculous regulation.

- The third reason that the draft regulation 2 is bad is that it is unenforceable. A regulation that cannot be enforced would be evaded, at first perhaps not in ways that would present any danger to health or safety, but

eventually by falling into general disrepute and perhaps being ignored totally. It is unenforceable because you would not be able to assess the mountain of forms that would descend upon you, and you would have grave difficulties in drawing a legal line between micro- and "macroorganisms". To enforce it rigidly would also require you to halt many quite "normal" human activities—for example the examination or treatment of people with X-rays (surely *likely* to alter the genetic constitution of microorganisms to which we are hosts), and the treatment of crops with chemicals (which even if they do not alter the genetic constitution of individual microorganisms will

surely alter the constitution of populations of microorganisms). Finally, it makes a nonsense of the evolutionary process: populations of all microorganisms are continuously changing their genetic constitutions and have been doing so since, quite literally, the origin of life. I fear that no law will stop this vast activity.

It is just not good enough to expect scientists to identify techniques (and organisms?) which may be excluded from regulation because they do not present "any potential hazard". Not only is the range of organisms studied so wide, it is impossible to prove that any human activity is completely safe under any circumstances. Dangerous

activities, on the other hand, can usually be positively identified. Whether you list activities to be excluded from the regulations or just those to be included, the regulations will have to be continuously amended to take the results of research into account.

It would be far more effective to draft the regulations to include only those techniques of *known* danger or which scientists judge to be of potential danger, and actively to involve both the scientific community and others in both the assessment of these dangers and the administration of the law itself.

Yours sincerely,

MICHAEL ASHBURNER

An open reply from the Director of the Executive

John Locke, Director of the Health and Safety Executive, takes up the points in Michael Ashburner's letter

Dear Dr Ashburner,

I am glad to have this opportunity of commenting on the points raised by you.

The Government and the Health and Safety Commission have both accepted what you call the "moderate" view that the recommendations of the Williams Report are reasonable. This means that the carrying out of the techniques described in shorthand as "genetic engineering" should be permitted where they offer prospects of social benefit, provided that adequate steps are taken to protect workers in the laboratories and people outside those laboratories from harm. The Williams Committee recommended that in order to achieve this protection those using these techniques, whether in official laboratories or in universities or in industrial firms, should be required to notify their intentions and to seek the advice of the Advisory Group which is being set up by the Government.

The proposals for Regulations, circulated for comment by the Health and Safety Commission, are intended to establish this requirement to notify. The Commission does not believe that it would be right in a matter of this importance to leave people free to decide whether to abide by the recommendations of the Williams Committee. They believe that everybody should be placed

in the same position of being required to give this notification.

The draft Regulations provide for notification to the Health and Safety Executive because we are a statutory body whereas the Advisory Group will have no statutory status. But I really must protest at the suggestion that the Advisory Group would play a subservient role to the Health and Safety Executive. This is simply not the case. It will be for the Advisory Group to lay down the conditions which need to be observed to enable the experiments to proceed safely. Our aim will be to make sure that the Advisory Group is in fact consulted, and secondly to make sure that its recommendations are followed. Both steps are necessary if workers and the general public are to be adequately protected and this is a responsibility placed upon us by Parliament.

Nothing in the Regulations therefore will remove the Advisory Group from its central role in the dialogue between those who wish to carry out experimentation of this kind and those who are competent to advise on the nature of the precautions which need to be taken.

The main point which you raise is that the proposed Regulations are drawn too widely and would catch many activities which do not present special hazards and which were not

dealt with by the Williams Committee. This is certainly true. But the Williams Committee clearly found it very difficult to produce a precise description of the kind of work which they wanted reported. And it is no good at all saying that scientists shall notify their intention of carrying out certain types of work unless they are told pretty precisely what is to be covered.

We hope that as a result of comments on the proposed Regulations, we may be able to suggest a definition of what is to be notified, which, as you say, will cover all those types of work which could present special hazards and require special controls, and which will not be exposed to some of the kinds of objections raised in your note. I entirely agree that neither we nor the Advisory Group should be subjected to "mountains of forms". On the other hand, I am sure you would agree that we must not devise a definition of what is to be notified which would leave out certain types of work which might prove to present special hazards. It seems to us we must err, if we err at all, on the side of having rather more than we want notified rather than too little.

I hope therefore that all those concerned in this field will help us with devising a definition of the activities to be notified to the Advisory Group and to my Executive which will be sensible, workable, and capable of offering the protection to which workers and the general public are entitled.

Yours faithfully,

J. H. LOCKE

Europe's drought (1)

Under the weather

R. M. Morris and R. A. S. Ratcliffe of the Meteorological Office, Bracknell, discuss recent anomalies of the weather and the general atmospheric circulation

THE drastic reduction in rainfall over much of the British Isles from early 1975 represented a rather sudden acceleration of a trend which began at the end of the 1960s. The average annual England and Wales rainfall for 1971–75 was 826 mm, the lowest mean five-year value since the 1850s. There have, however, been six five-year periods since 1825 with annual averages less than 840 mm, so the recent five-year rainfall may be expected to occur once in about 25 years.

Rainfall in the period May 1975 to August 1976 was exceptionally low—in fact the driest such period in England and Wales since 1727, when records officially began. The rainfall total for this period is provisionally assessed at 760 mm compared with the next lowest value of 809 mm in the eighteenth century. Records are regarded as unreliable before about 1825 and if one excludes years before that date the next driest 16-month period starting in May was in 1933–34 with a total of 900 mm. It is difficult to calculate return periods of such an unusual event, but the standard formula suggests at best a once-in-500-year occurrence.

In England the period 23 June to 8 July, 1976, was also exceptionally warm. Mean daily maxima were some 10 °C above the normal values for the time of year and reached 32 °C in some places. Such an event is also unprecedented over the past 250 years. These high temperatures were partly due to the very dry ground, which meant that a smaller proportion of incoming solar radiation than usual was used for evaporating moisture; more was thus available for heating the ground and hence the atmosphere.

Broader perspective

Despite the severity of dryness in England it is important to view the anomalies in a broader spatial and temporal perspective. The distribution of rainfall over most of Europe for the period October 1975 to July 1976, expressed as a percentage of the long-term average (see map), shows that the area of less than 75% broadly extends southwest from Sweden, across the low countries and France to northern Spain, embracing much of England, Wales, eastern Scotland and also southern Germany. Less than 50% is confined to southern England

and northern France with minimum values near the coast of central southern England. On the other hand above average rainfall has occurred over Norway and extreme northern Scotland probably extending across Iceland, whilst similar or greater excesses are evident over western Russia, the Balkans and southern Italy.

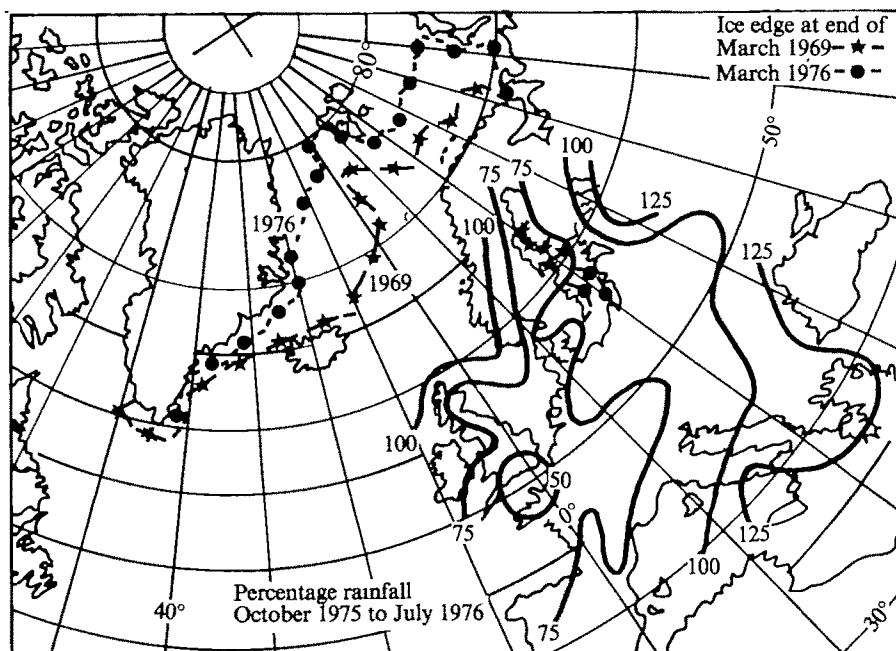
The distribution and evolution of rainfall anomalies on the scale shown is closely linked to the evolution of the major circulation systems in the tropospheric flow around the northern hemisphere. In particular the jetstreams (that is, zones of strong quasi-horizontal wind flow in the middle and upper troposphere) are usually closely associated with the zones of strong thermal contrast which separate regions of markedly different temperature and humidity characteristics. Jetstreams are intrinsically associated with weather because they represent a major cause of the upward and downward motion of the air in the middle layers which produces both extensive cloud systems and cloud-free regions in the circulation. The presence of a jetstream above a certain region usually implies changeable weather, with periods of rain alternating with periods of fine weather. Regions on the cold side of the jetstream (usually the northern side) tend to have a good deal of rain and showers; regions on the warm side tend to have a predominance of dry weather.

Within the past two years the location of the mean jetstream in the Atlantic–European sector of the circulation has shifted almost 1000 miles northwestwards in a coherent and continual manner. The second map illustrates the movement of July–August mean mid-tropospheric jetstream flow from 1974 through 1975 to 1976. Relatively small changes have occurred in the St Lawrence–Newfoundland area and also across the Mediterranean, and a major change has occurred between the northwest European seaboard and Iceland. To put matters in perspective, the mean summer position of the jetstream over the period 1951–70 was close to latitude 50°N across the Atlantic and extended rather weakly to the English Channel. With the jetstream in this position English summers tend to be rather cool and unsettled.

Jetstream shifted

During the winter of 1974–75 the main jetstream across Britain shifted to a position just northwest of the Hebrides. Apart from a temporary falter during the spring it maintained this position throughout most of 1975, moving a little further northwest during November and December. Despite some extremely vigorous cyclonic activity in the northwest Atlantic early in 1976, which actually encroached upon western parts of the British Isles during May, the mean jetstream clung tenaciously to a position between Iceland and Scotland.

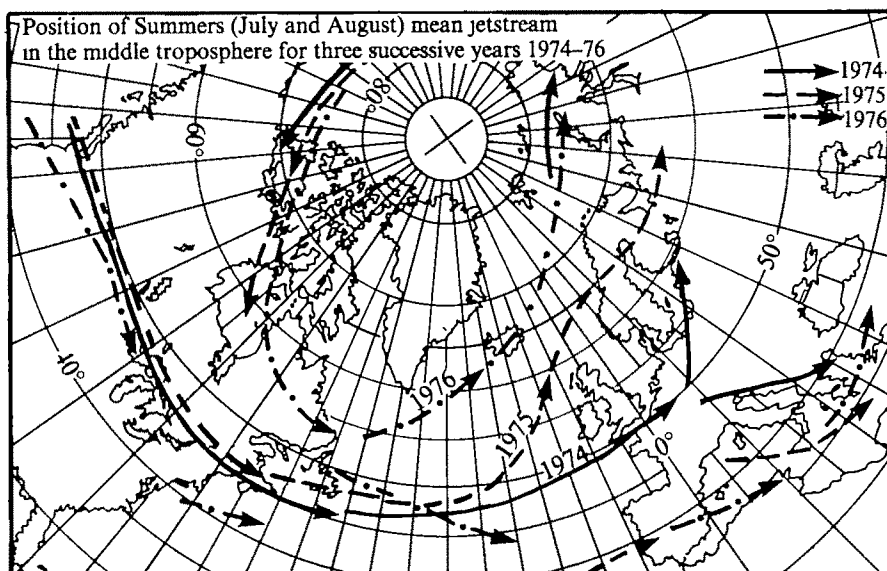
Following the recent summer the jetstream cannot extend further north in view of the proximity of the Arctic ice fields, which will inevitably extend southwards in the winter. The distribution of temperature gradient in the ocean–atmosphere system seems certain



to develop a new jetstream from the eastern USA across the Atlantic towards western Europe, thereby fundamentally changing the situation. Indeed this has already occurred and has been responsible for the recent heavy rain over much of the area worst affected by the drought.

The movement of the mean jetstream flow well to the north of the British Isles was the major cause of the marked reduction in rainfall, and the excesses of rain further north were clearly associated with an increase of jetstream activity not normally present in those latitudes. At the same time the influence of a jetstream upon rainfall anomalies is of limited geographical extent and such is the nature of the circulation that the predominance of cyclonic or anticyclonic regions in a particular region usually leads to the development of anomalous regimes in an adjacent region. The excess rainfall over the eastern and southern fringes of Europe is probably associated with this kind of circulation compensation.

The overall trend during the 1970s has been one of increased cyclonic activity in the Arctic basin, especially across the extreme north Atlantic and



Norwegian Sea, and increased anticyclonic conditions over western Europe. Such a circulation implies more frequent and stronger westerly winds than usual north of about 55° N in our sector. The increased westerly winds and cyclonic activity in the north have been associated with a marked recession northwards of the Arctic ice.

Up to 1969 Arctic ice to the north of Britain was extending southwards and reached its extreme southern position in March 1969 (see map). By March 1976 the area of open water in the Greenland-Spitzbergen area had increased by about 1.4 million square kilometres compared with March 1969. □

Europe's drought (2)

Water everywhere?

Haydon Richards of the Central Water Planning Unit assesses Europe's water resources

WATER is one of the natural resources with which Europe is better endowed than many other large areas of the world. This generalisation does, however, mask variations in geographical distribution of resources as compared with demands. The summer of 1976 has emphasised an extreme of variation. The driest 16-month period on record, from May 1975 to August 1976, has severely tested resources designed to provide a continuous water supply, but on the whole demands have been met and criteria used for design have proved to be sound. If living conditions are to be maintained or improved then demands have to be met for the four main sectors of public water supply, industry, agriculture and thermal power station cooling. The unusual conditions of 1976 have at least emphasised those areas where additional storage of water in some form needs to be made available in order to satisfy these demands and not impose severe restrictions on water use.

Estimating demand

Reasonably accurate estimates of

demand need to be made in order to plan development of suitable resources. Traditionally, trends over a number of years have determined the level of demand to be met at a particular time in the future, but more recent estimates have attempted to break down the components of the total demand. A good deal still remains to be done in this field. Publicity this summer has led to marked lowering of demand for public water supplies and for industry, amounting to at least 20% of normal demand in some areas. However, the knowledge that water is again abundant after a time may well reduce the attitude of care and thus increase demands to "normal" levels.

Apart from the demands to be met from the four major sectors the needs of ecology and amenity have to be borne in mind. Sufficient water must be left in rivers and lakes for aquatic life to continue, effluents to be suitably diluted, navigation to be possible and for fishermen and picnickers to enjoy their leisure. There must therefore be some idea of the quantity to be left in a natural watercourse to prevent un-

acceptable damage to the environment. This quantity can be determined only by agreement between all users of a particular river catchment and the criteria used may differ from one to the other, general amenity perhaps being over-riding in an upland or rural setting and effluent dilution in an industrial area.

Future projections in demand for water until the end of the century suggest high growth rates in excess of 200% in the Netherlands and at least 150% in Luxembourg, whereas in France demands may increase by little more than a quarter. Recent estimates indicate that Germany and the United Kingdom will need to increase use by 60 or 70%. Those countries with a high proportion of agricultural water demands locally, such as France and Italy, may have developed methods of meeting those demands locally, whereas the increased industrial demands in other countries have to be met by providing major new resources at some distance from the centres of demand.

Increasingly, demands for cooling water are being met from saline waters. Italy and Denmark benefit from long coastlines and use only a small proportion of freshwater for cooling in thermal power stations, although thermal pollution then becomes a problem. Some 30% of cooling tower water is evaporated, and this forms a consumptive use of water because it is lost as a resource. In the same way

water used for irrigation is almost totally lost as a water resource. Groundwater is important for meeting demands for potable water because it is usually of good quality, but only in France is it used to supply water for power station cooling. In Denmark, where there are no rivers of any size, nearly all water for public supplies, agriculture and industry is derived from groundwater storage, and in Belgium about three-quarters is so derived; in Ireland very little is used.

Water resources

Water resources are renewed annually from precipitation on the land surface, the effective rainfall being that which remains for surface runoff or infiltration to groundwater storage after evaporation and replenishment of soil moisture deficits.

Calculating average annual renewal of water resources provides an idea of the potential resource available in different countries. Ireland with its moist equable climate has approximately 32,000 litres a day available for each person from effective rainfall, whereas Belgium has about 3,000 litres a day; figures for other countries in north-west Europe lie between these.

The daily per capita consumption for all purposes in 1969 in Belgium was 119 litres and other countries range up to 280 litres. Even allowing for minimum flows to be maintained in rivers, therefore, nature still provides a substantial surplus of water which can be exploited to meet changing or increasing demands. Belgium's fresh water resources are found in three rivers and in extensive aquifers, and from the latter is obtained almost three-quarters of the public water supply. Regarding other European countries:

- On the low-lying Danish peninsula the small streams are fed by natural groundwater discharge. Some surface water is used to supplement the Copenhagen supply, but groundwater supplies nearly all the country's needs apart from thermal power plant cooling.

- Rainfall in France varies from about 850 mm per annum in the west to less than 500 mm near the Mediterranean. Below average snowfall in the Alps last winter slightly reduced some stream-flows during the summer of 1976. There are extensive aquifers in north east France and alluvial aquifers in the major river valleys.

- In West Germany there are also several rainfall zones, from the alpine south with high rain and snowfall, through the central highlands where the average annual rainfall is at least 1,000 mm, to the central lowland with an annual average of 730 mm. The flows of all the main rivers are fairly uniformly distributed throughout the year. Groundwater occurs mainly in the

flat alluvial tracts of the river valleys and beneath the northern plains, and provides well over half the public water supplies.

- Ireland is better endowed with water resources than most other countries in Europe, having a low population density and high rainfall which varies from 2,000 mm per annum on the west coast to little over 600 mm on parts of the east coast. There are several rivers and numerous lakes. Groundwater is widely used to supply farms but this source accounts for only about 7% of the total demand for potable water.

The Rhine is of first importance to water management in the Netherlands and it provides over 60% of the total, including power station cooling; groundwater provides more than a quarter of this total and the remainder is derived from the Meuse and small rivers. Navigation requirements also exercise control over water use because minimum levels have to be maintained to meet international agreements. Any open connection with the sea creates the possibility of an inflow of salt water and so substantial outflows are required in the areas below sea-level in the west of the country. Such control is linked to the general care taken to control salt water seepage into the polder areas. Potential resources from the Rhine are very large, but because of variations in the quality of the water management problems in the Netherlands are complex and involve storage in lakes, artificial recharge of sand dunes and the linking of existing sources.

There are marked geographical variations in rainfall in the United Kingdom; annual variations have been highlighted recently with rainfall in the year to August 1976 only 63% of the long term average over England and Wales. Groundwater provides 35% of public water supplies in England and Wales. Natural groundwater discharge provides 80% or more of the total annual flow of many rivers flowing from or over the major aquifer, the Chalk. Lesser percentages are contributed to streams from other aquifers.

Traditionally in the United Kingdom demands have been met in most areas by direct pipeline supply from upland reservoirs; reservoirs in the lower Thames valley are filled by pumping from the river. Along several rivers in England, water is used, returned as treated effluent and re-used lower down stream, and advances in the technology of water treatment in the last few decades has increased the possibility of using upland storage to regulate the flows of such rivers.

Water management

Both Ireland and Denmark tend to use a water resource and then ensure that the effluent is discharged after suitable

treatment to the sea. Countries with large inland industrial and urban areas such as France, Germany and the United Kingdom are faced with the problems of costly effluent treatment if the environment is to be protected, and particularly if water is to be re-used for further public, industrial or agricultural supply or amenity. In France, as in Italy, there are large numbers of small reservoirs in the hilly areas providing storage for farm irrigation, but this is not common elsewhere. Systems of control over agencies supplying water, managing rivers and dealing with effluent and sewage treatment vary greatly, but most countries have some form of statutory or voluntary means of tackling aspects of water development and use in defined catchments.

Direct supply of water from source to demand area will continue, but study of the relative costs of providing additional resources will probably produce different methods of using some existing sources. Schemes for the combined use of a number of sources can exploit their contrasting temporal characteristics, as for example when a supply from a river during winter is stopped during summer at times of low river flow, the summer supply being obtained by abstraction from groundwater storage which in turn is normally refilled by winter infiltration from rainfall. Such complex operations require suitable systems of data collection relating to quantity and quality of water in storage above and below ground, and the ability to forecast resources in store, changes in demand, operational problems and the likely results of any decision to use resources differently.

In spite of the uneven distribution of rainfall and of resources in rivers, lakes and aquifers there is sufficient water in the countries of north west Europe to satisfy foreseeable demands well into the next century. Care will be needed to ensure correct treatment of effluents, protection of the quality of surface water and groundwater and the maintenance of river flows for navigation and amenity. Additional storage is needed to meet shortages in certain areas, and by and large these have been emphasised during the dry period of the summer of 1976. This year has also shown that many of the major reservoirs have been designed so as to provide a large part of the required supply even through these unusual conditions. Groundwater storage has also helped by allowing abstraction at rates in excess of the long-term average renewal of storage, and the potential for even greater use during another severe dry period should be determined in necessary detail. No doubt methods of influencing demand patterns and modifying demands will be explored and developed. □

POPULATION

Will world population double?

Colin Norman looks at a new analysis of population trends throughout the world which has produced findings that are both encouraging and decidedly grim

THERE has been a dramatic slowdown in world population growth in the past five years as many countries, paced by China and the United States, have brought their birth rates down sharply. But death rates have risen steeply in some poor countries because crop failures and increasing pressure on agricultural resources and fisheries have claimed about two million lives. So says the study* of population trends conducted by Lester Brown and published by the Worldwatch Institute of which he is the President.

It paints a grisly picture of a world living from hand-to-mouth, with virtually no food reserves left to guard against poor harvests or other natural disasters, and with croplands, forests, grasslands and fisheries in many poor, densely populated regions close to breaking point. Brown concludes from the trends that "it's quite possible that we will never again see a doubling in world population growth, in spite of the fact that it is the standard rhetoric of UN officials", because for a great majority of countries, "a doubling of population will yield potentially unmanageable ecological, economic and political stresses".

Brown's principal conclusion is that "sometime near the beginning of this decade, the rate of world population growth reached an all time high and then began to subside". Though the total number of people in the world rose from 3,590 million in 1970 to 3,920 million in 1975, Brown estimates that the annual rate of increase has shrunk from 1.90% to 1.64%, a dramatic and, to some observers, surprising decline.

The bright side of the picture is that birth rates are falling in almost every country as more and more governments are reversing pro-natalist policies and instituting family planning programmes. Four European countries—East Germany, West Germany, Luxembourg and Austria—have already reached zero population growth and two others, Belgium and the United Kingdom, are expected to reach that point sometime this year. But by far the most dramatic reduction has

occurred in China and in some other East Asian countries.

With one-fifth of the world's people, China has a huge influence on overall population trends. According to Brown's estimates, China has just achieved "family planning's greatest success story", reducing the country's birth rate from a crippling 32 per 10,000 in 1970, to a more manageable 19 in 1975. Though hard and fast data for China are difficult to come by, Brown bases his estimate chiefly on figures supported by a variety of sources, on birth and death rates in political units in China, which he extrapolates to the entire population. His estimate for China's population growth is lower than one produced by the US Bureau of the Census a couple of years ago, but higher than a more recent estimate of the Agency for International Development.

But China isn't the only country to show a surprising reduction in its population growth. In some ways, the trend in the United States is equally striking. During the past five years, the growth rate in the US has declined by about a third, from 0.9 to about 0.6%, Brown estimates, in spite of the fact that children of the post-war baby boom have recently entered their prime reproductive years. The reasons include a drop in the marriage rate, increasing employment of women, and increasing female enrolment in graduate and professional schools.

And social trends elsewhere are equally significant. Mexico has recently completely reversed its pro-natalist policy and has instituted a strong family planning effort. And abortion laws have recently been liberalised in many countries, including predominantly Catholic nations such as France. In fact, Brown states that the proportion of the world's people living in countries where abortion is easy to obtain has increased from 38% to 64% in the past five years. "Few social changes have ever swept the world so quickly", he notes.

But in some parts of the world rising death rates have also played a part in reducing population growth. The grimest figures have come from the Indian sub-continent, particularly Bangladesh. Though Brown notes that few national leaders are eager to discuss rising death rates and good data are therefore difficult to obtain, he has pulled together data from a variety of sources which put into perspective the tragic impact of the Bangladesh war of independence from Pakistan and the

failure of the Indian harvest in 1972.

Brown bases his estimates for Bangladesh on meticulous records for death rates in the province of Matlab Bazar, maintained by the International Cholera Research Laboratory. The death rate in that province climbed from 15.3 in 1966-70 period to 21.4 in 1972. If the figure is extrapolated to the entire country, it suggests a nationwide increase in deaths from hunger of 427,000 that year. Similar extrapolations for 1974-75, when Bangladesh's rice crop was poor, suggest that the grim death toll was about 330,000.

Equally distressing figures emerge from extrapolations in India. In 1971 and early 1972, India had fed an estimated 8-10 million refugees from Bangladesh from its own grain reserves, but in late 1972 it was caught by two related disasters. First, the monsoon failed that year, severely reducing India's wheat harvest. And second, that same year, the Soviet Union imported 30 million tons of grain from the United States, thereby tying up most of the world's exportable wheat supplies. Official figures for death rates in India that year show an increase in Uttar Pradesh from 20.1 to 25.6, and equally steep jumps in two other provinces. Brown calculates that "in these three states alone, hunger claimed an estimated 829,000 lives". The figures in the Worldwatch report are the first authoritative estimates of the impact of crop failures on demographic trends in the region.

But Brown argues that death tolls from massive failure of crops and other disasters such as the Sahel drought are only one measure of the impact of overpopulation. Less obvious, but potentially more important is the fact that population growth is "simultaneously contributing to growth in food demand and to reduced food output". Overfishing is depleting fish stocks, he argues, and in many regions, overgrazing, deforestation and overploughing are leading to soil erosion, desert encroachment and the abandonment of crop lands. "It has been evident for some time that oceanic fisheries could collapse under the pressure of excessive demand. What is becoming equally clear is that land-based food systems can also give way under intense pressure", he argues.

For those reasons, he contends that it is difficult to believe that world population will ever double again, simply because few countries are capable of supporting double their present population.

Such realisations are beginning to show up in the implementation of family planning programmes. Nowhere is that development more striking than

**World Population Trends: Signs of Hope, Signs of Stress*, published by the Worldwatch Institute, 1776 Massachusetts Ave NW, Washington DC 20036. \$2.00.

in India, perhaps the nation hardest hit by hunger in the past few years. In the state of Maharashtra, the legislature has approved with only one dissenting voice a bill for compulsory sterilisation

of all males with three or more living children. Even if the measure is never implemented, the fact that such Draconian steps are being discussed is an important indicator of the serious-

ness with which India now regards the population problem. "Five years ago", Brown said last week, "compulsory sterilisation would not have been an acceptable topic for discussion in India".

NUCLEAR TRADE

Ford makes his move

With just five days to go before the Presidential election, Mr Ford last week announced long-awaited proposals designed to prevent countries which import nuclear technology from building atomic bombs. Colin Norman reports from Washington

MR FORD's proposals, which have been extensively leaked during the past few weeks, represent some significant reversals of past Administration policy, and in some respects resemble less detailed suggestions made last May by his Democratic opponent Jimmy Carter. The crux of the new policy, outlined in a lengthy statement released by the White House, is that the United States should back away from its own plans to separate plutonium from spent nuclear fuel and recycle it as a reactor fuel. In addition, Mr Ford offered a raft of proposals for international co-operation in preventing the spread of nuclear weapons, including the following:

- He called upon all countries to refrain from selling reprocessing technology or uranium enrichment plants for at least three years. Already, West Germany has agreed to sell reprocessing and enrichment plants to Brazil, and France is committed to selling a reprocessing plant to Pakistan. Asked last week whether the Administration intends to enforce the policy in respect of those two deals, the Under Secretary of State for Economic Affairs, Charles W. Robinson, hedged by suggesting merely that "I think the statement that has been released by the President will be helpful in pursuing our interests in this matter".

- Mr Ford urged "new co-operative steps . . . to help assure that all nations have an adequate and reliable supply of energy for their needs". To that end he has asked the Secretary of State to initiate discussions with other suppliers to seek arrangements for co-ordinating fuel services. In particular, "these discussions will address ways to ensure against economic disadvantage to co-operating nations and to remove

any sources of competition which would undermine our common non-proliferation efforts". He also announced that the United States should increase its own enrichment capacity, particularly by expanding the government-owned plant at Portsmouth, Ohio. (The statement, it should be noted, was made in Cincinnati, Ohio.)

- Ford also stated that he has asked the Secretary of State to initiate international discussions aimed at establishing a new facility for storing plutonium and spent fuel from civil power stations, under the control of the International Atomic Energy Agency. He also pledged to place excess US plutonium in the facility once it is established.

- As for sanctions to deter nations from acquiring nuclear weapons, Ford stated that "any material violation of a nuclear safeguards agreement . . . must be universally judged to be an extremely serious affront to the world community, calling for the immediate imposition of drastic sanctions". In particular, he promised that the United States will, "at a minimum" respond to violation of any safeguards agreement to which the US is a party with "an immediate cutoff of our supply of nuclear fuel and cooperation to that nation".

In that regard, it should be noted that the atomic device exploded by India in 1974 was made from plutonium produced in a reactor which was moderated by heavy water supplied by the US under an agreement that it be used only for peaceful purposes. Does Mr Ford's threat to cut off fuel supplies therefore apply to India's apparent violation of its safeguards agreement? Mr Robinson would not commit himself last week, noting only that the United States has been negotiating with India to repurchase spent fuel from the Tarapur Atomic Plant, and that those negotiations "will be given new emphasis and support" by Ford's statement.

Asked later whether a second explosion by India would be sufficient to prompt a cutoff in aid, Robinson still refused to be drawn: "That obviously

would be viewed as a very serious matter . . . We must understand, however, that we are going back to an agreement concluded a number of years ago where our present concerns were not fully reflected in the contractual terms".

As for the United States' domestic nuclear programme, Ford said that he would speed up the effort to find a suitable method of disposing of radioactive wastes, and that he is directing the Secretary of State to initiate international discussions on the possibility of establishing centrally located, multinational waste repositories. His most important announcement, however, was that the US nuclear industry should no longer plan on reprocessing wastes and recycling plutonium.

A commercial reprocessing plant is already almost completed in Barnwell, South Carolina, and the nuclear industry has applied for a licence to begin recycling plutonium as a reactor fuel. Ford stated, however, that the United States "should no longer regard the reprocessing of nuclear fuel as a necessary and inevitable step." Reprocessing and recycling, he said, should only be permitted "if they are found to be consistent with our international objectives".

That leaves the Barnwell plant in a peculiar position since a decision to forego reprocessing would make it redundant before it even starts operating. One possibility, however, is that the Energy Research and Development Administration may seek Congressional approval to complete the plant and to operate it on an experimental basis to help evaluate the economics and the technological and safety problems associated with reprocessing. Ford's statement, in fact, said that he hopes to seek international participation in such an evaluation effort.

Finally, it should be noted that Ford's statement made no mention at all of an aspect of nuclear proliferation which many arms control experts see as central to the issue, namely, the controls which the superpowers are willing to place on their own weapons developments, particularly the testing of nuclear weapons. But those are touchy issues, and it was five days before the election. □

SWEDEN

● Less than a week after the formation of Sweden's new three-party government, Prime Minister Thorbjörn Fälldin's election promise to stop the insertion of fuel into the Barsebäck 2 nuclear reactor fell victim to the compromises said to be necessary for the survival of the coalition and insertion was allowed to go ahead. In a declaration of the government's programme, Fälldin said in effect that the government would stop the reactor going into operation unless its owners produce acceptable plans for the reprocessing of spent fuel and proof that radioactive waste will be stored in complete isolation from all life for eternity. The deadline for the presentation of these plans is October 1, 1977.

Politicians opposed to nuclear energy assert that the reactor's owners will not be able to come up with any proposals acceptable to the government. The nuclear industry is confident that it can. The effect is to throw open the entire question of whether the country's reactor plans are to be stopped, or even greatly slowed down: Sweden's construction workers are already seeking assurances about nuclear power stations now being built.

Fälldin also announced the formation of a commission which will evaluate the research done so far on the security and environmental aspects of nuclear power as well as the storage of highly radioactive waste. The commission is also to make suggestions for a new energy policy to be presented to parliament in 1978, until which time it seems that the futures of other reactors under construction will hang fire. It has at least been made clear that the twelfth in the planned series of thirteen reactors will not now be built at all, and this would presumably also apply to the thirteenth.

Meanwhile, in what has been dubbed as Operation Striptease, the findings of the Committee on Radioactive Waste have been attacked by academics and politicians who want to reveal its unwarranted optimism about the long-term storage of spent fuel. Action to endorse the committee's conclusions has come from only one source, a local government council which, amidst lively public debate, has voted to allow reprocessing and storage plants to be built near the Forsmark reactors already in operation.

● The hullabaloo over Barsebäck 2 has overshadowed a surprisingly favourable legal decision recently won

by one anti-nuclear power group. The Supreme Water Court has ordered the State Power Board to lower the level of radioactivity in the cooling water to be discharged from the third and fourth Ringhals reactors. In addition, the Court has directed the Board to pay about US\$22,000 in legal



costs to those who pressed the case, the environmentalist Björn Gillberg and Gunnar Michelson, a lawyer.

Following the Court's decision, the release of tritium must be lowered from 10,000 to 3,700 curies per reactor per year; of isotopes emitting beta and gamma rays, from 100 to 2–24 curies per reactor per year; and of isotopes emitting alpha rays, from 50 to zero curies per reactor per year. As the case of beta and gamma rays suggests, the new figures are not so much absolute limits as indications of what the Court thinks should be feasible, according to what is technically possible and economically reasonable. Its decision allows the Power Board to make very occasional releases of much greater amounts of radioactivity—but only when it may be technically impossible to avoid doing so. Any repeatedly greater releases would be investigated by the National Institute for Radiation Protection and could put the Board back in court for another trial.

Gillberg and Michelson lost that part of their case in which they had argued for a reduction in the volume of cooling water which is to be released into the sea. According to Gillberg, they are thinking of appealing on this issue to the Supreme Court. If they do, they will shore up the Prime Minister's creaking campaign to stop Sweden's nuclear power programme. The reactors' debut (planned for December 1977 and July 1979) would have to be postponed until the appeal had been heard and a judgment made, a process which would probably take two or three years.

● With nuclear fission such a controversial issue, the case for fusion could grow. Bo Lehnert, professor of plasma physics and fusion research at Stockholm's Royal Institute of Technology, says fusion would not involve the two problems normally associated with fission, namely radioactive waste products and reactor accidents; the radioactivity which neutrons would induce in the material surrounding the reaction could be diminished by using vanadium and its alloys in the wall material. He says that even if materials less suitable than these were used, the level of radioactivity and the corresponding biological hazards would be many orders of magnitude smaller than with a corresponding amount of energy produced by fission. Reactor accidents would also be less severe with fusion than with fission, says Lehnert, partly because no runaway nuclear reactions would be possible in an incident involving a fusion reactor.

Fusion power, then, is extremely attractive to the Swedes, as it is in many developed countries, and they have not been idle in their research. Their interest dates from the mid-1950s, Lehnert's group being financed mainly by the Swedish Atomic Research Council and the Bank of Sweden Tercentenary Fund. From 1976, however, it has joined with the team working under Hans Wilhelmsson, professor of electromagnetic field theory at Chalmers Institute of Technology, near Gothenburg, to form a fusion research unit associated with Euratom, which now shares the support of the unit with the Atomic Research Council. The immediate result of this has been a doubling of research funds, which amount this financial year to Skr4 million (about US\$900,000).

Lehnert is, however, dissatisfied with what he considers to be the narrowness of present fusion research. His general, and personal feeling—"controlled optimism for controlled fusion"—cannot give immediate comfort to anyone involved in the debate over fission reactors. Given a frontal attack on a wide variety of problems, including basic research, new ideas and modified old ideas, he believes that commercial fusion reactors may be "not quite unimaginable" before the year 2000. But with research going in current directions at its present level of finance, he forecasts a longer wait.

Wendy Barnaby

IN BRIEF

Ford statement attacked

President Ford was given a sharp rap over the knuckles last week by 10 Nobel prizewinners and one former Presidential science adviser for trying to make political capital out of the fact that the United States made a clean sweep of this year's Nobel prizes. Presenting the National Medal of Science to 15 American scientists last month, he took the opportunity to score a few political points, and without mentioning Jimmy Carter by name said that the Nobel sweep would "surely put to rest" suggestions that the United States had lost respect around the world. He then argued that there is a need to "bolster research and development to achieve national goals".

The remarks, and their implication that the Administration should share the credit for the Nobel awards, were attacked in a statement drafted by George Kistiakowsky, professor of Chemistry at Harvard and former science adviser to President Eisenhower, and signed by 10 Nobel prize-

winners including the winner of this year's award for Chemistry, William Lipscomb. The statement noted that "Nobel Prizes usually reflect work done over long periods of time. This year's prizes do not, therefore, reflect this year's strengths". The statement went on to argue that Mr Ford's budgets "have not been such as to encourage the growth of American science. The current appropriation for the National Science Foundation is actually 10% lower than it was in the year when Mr Ford took office." Other signatories were Kenneth Arrow, Julius Axelrod, David Baltimore, Owen Chamberlain, Carl Cori, Donald Glaser, Salvador Luria, E. M. Purcell and George Wald.

ESF optimism

At the conclusion of the European Science Foundation's second annual general assembly in Strasbourg last week the president of its Executive Council, Sir Brian Flowers, said he detected the emergence of a single European voice in science. The

assembly had accepted the findings of ad hoc committees on genetic manipulation and astronomy.

One material consequence is that a European committee will be established under the aegis of the ESF both to discuss recombinant DNA research and to keep under continuous review the guidelines covering it. The committee will consist of representatives of the various national advisory bodies concerned together with representatives from both the EMBO Standing Advisory Committee and the European Medical Research Councils.

Acceptance of the astronomy committee's recommendations means that Europe's instrumental resources will soon be concentrated on a mountain ridge of La Palma in the Canary Islands. The first major step would involve moving the British Isaac Newton Telescope; Sweden's instruments now at Anacapri in Italy would follow, and a new Austrian telescope would come later. The Observatory itself would be Spanish.

At 10.30 a couple of Sundays ago I cut a tape across a footpath and led a crowd of 361 men, women and children on a sponsored walk around Grafham Water, one of the largest man-made lakes in southern England. Our purpose was to raise money for the Ouse Valley Wildlife Appeal to enable the Bedfordshire and Huntingdonshire Naturalists Trust, a voluntary conservation organisation of which I am president, to buy land for nature reserves. Almost all the walkers completed the modest circuit of ten miles, and so called upon the maximum generosity from those who had agreed to support the appeal, at rates ranging from a halfpenny to a pound a mile. My own efforts produced £56.20; the whole exercise raised more than £2,000. The Trust's last acquisition was Upwood Meadows, 14 acres of botanically interesting meadow probably unploughed since the Black Death; it cost us £5,000, so the walk paid off the debt on about five acres.

England is one of the most crowded areas in the world. The growing population, industry, affluence and increasing mobility are putting intense pressure on wild plants and animals and on the habitats which support them. Modern agriculture is changing the look of the countryside, and the wholesale removal of hedges, rows and copses means that many birds, butterflies and flowers which were formerly common and widespread are difficult for the non-expert to find. Sites for scientific study, for

education and for ecological research are disappearing.

Fortunately there are in Britain official bodies like the Nature Conservancy Council and the Countryside

Walks for wildlife**KENNETH MELLANBY**

Commission which are charged with conserving wildlife and rural amenity, and this they do admirably to the limit of their meagre resources. It is encouraging that the Nature Conservancy Council has been able to designate an increasing number of outstanding National Nature Reserves in recent years, by purchase, lease or "nature reserve agreement". This last system is one by which liberally-minded landlords agree to restrict their activities so that the area will

retain its scientific importance. It is feared, however, that new legislation may compel some such landlords or their heirs to sell their property, and that the buyers may be less willing to give priority to a few rare orchids or to wildfowl.

It is therefore fortunate that official efforts are so often supplemented, and sometimes surpassed, by the many voluntary conservationist organisations in Britain. Critics may complain that conservationists are mainly a middle class elitist group, and this is to some extent true—and why not—but they are making great efforts to bring all sections of the population into their ranks.

Many of the reserves established by County Naturalists Trusts are conveniently situated to be used as open-air classrooms by local schools. When the pupils become involved in strenuous management exercises, such as removing unwanted scrub which is crowding out the more exciting plants, they may become converts to conservation. Even sponsored walks involve a new section of the population, and if, as at Grafham, these go through reserves with simple nature trails marking clearly some of the points of importance, the walkers' temporary interest may be made permanent. It is estimated that nearly five thousand of those who sponsored the walkers were not otherwise concerned with conservation: perhaps the success of the venture will turn their sympathy to real interest and support.

news and views

DNA sequences coding for more than one protein

from Benjamin Lewin

THE single strand DNA phages of *Escherichia coli* are amongst the smallest viruses known (only the RNA phages are appreciably smaller) and are therefore of special interest for the strategies evolved by their genomes to allow successful reproduction with such limited genetic information. In fact, a paradox in the organisation of their genomes has recently been suggested by the identification of a greater number of phage-coded protein products than it appears can be accommodated within the complexity of the genome. Both types of single strand DNA coliphage possess a circular genome, in the case of the icosahedral phages ϕ X174 and S13 fairly well characterised as about 5,500 bases, and for the filamentous phages f1, fd, M13, apparently somewhat larger.

A genome of 5,500 bases should have a maximum coding capacity equivalent to some 200,000 daltons of protein. But measurement of the proteins induced by infection with ϕ X174 identifies a total of nine species, with a combined weight approaching 250,000 (Benbow *et al.*, *J. Virol.*, **10**, 99; 1972; Godson, *J. molec. Biol.*, **57**, 541; 1971). Each of these protein products can be equated with one of the nine identified genes of the phage. In spite of some uncertainties about the sizes of two of the proteins, it is clear that their total mass is appreciably greater than is to be expected of the phage genome.

One mechanism utilised by the phage is to produce two proteins from one gene. Products of gene *A* can be identified with weights of about 60,000 and 35,000 daltons, and Linney and Hayashi (*Nature new Biol.*, **245**, 6; 1973) showed that the smaller protein represents the C-terminal part of the larger. Probably it results from an internal initiation of translation about halfway along the gene. The functional relationship between the two products is not clear.

A situation more difficult to resolve has been presented by the genes *D*, *E* and *J*, whose products have been identified as a protein of about 15,000 daltons needed for particle assembly, a protein of about 17,500 daltons needed for lysis,

and a particle component of about 9,000 daltons. The construction of a restriction fragment map for ϕ X174 suggested that there could not be room for all three genes in the part of the genome where their mutations lie. Restriction maps of ϕ X174 DNA have been developed with several different enzymes, including *Hind*II, *Hae*III and *Hpa*II; calibrating the sizes of the fragments with known standards generated a total length for the genome of 5,200–5,400 bases, slightly less than the previous estimates of 5,500 (Lee and Sinsheimer, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2882; 1974). By using a marker rescue technique, Weisbeek *et al.* (*Virology*, **72**, 61; 1976) recently were able to locate mutations on to *Alu*I, *Hind*II or *Hae*III fragments, and thus to calibrate the restriction map with the genetic map. This allowed the maximum possible size of each gene to be calculated, and in most instances this suggested that the protein products must be slightly smaller than the sizes previously estimated. But in the region where genes *D*, *E* and *J* lie, the sizes of the restriction fragments exclude the possibility that there might be three contiguous genes coding for proteins totalling more than 40,000 daltons. This seemed explicable only in terms of the postulate that mutants thought to identify *J* might in fact reside in *E*, eliminating the *J* gene from the map, but at the same time raising a question about the origin of the *J* protein and its relationship to the *E* protein.

In a striking demonstration of compact genetic organisation, Barrell, Air and Hutchison (page 34 of this issue of *Nature*) now resolve this problem. Using the "plus and minus" technique for DNA sequencing developed by Sanger and Coulson (*J. molec. Biol.*, **94**, 441; 1975), they have determined the sequence of genes *D*, *E* and *J*. (In fact, the entire sequence of ϕ X174 DNA has also been determined and is quoted as not more than 5,400 nucleotides, confirming the limits on genetic complexity previously established, and in good agreement with the total lengths of the restriction fragment

maps.) The sequence of the *D* gene was unequivocally correlated with its product by determining the sequence of the protein. The termination codon of gene *D* overlaps the initiation codon for gene *J* by one nucleotide: thus in the sequence TAATG, the first three bases provide the ochre codon terminating gene *D* while the last three bases provide the initiator for gene *J*. The following sequence can be defined as gene *J* by virtue of its equivalence with the amino acid sequence of a small protein isolated from the virion, which presumably corresponds to the product previously identified with *J*. However, the *am6* mutation that had been taken to define *J* genetically lies 179 nucleotides before the initiation codon for *J*, substantiating the suggestion of Weisbeek *et al.* (*op. cit.*) that it lies in another gene.

Where does gene *E* lie? Marker rescue experiments identified the location of two mutations, the classical mutation *am3* that has been taken to identify gene *E*, and the mutation *am6* that Weisbeek *et al.* suggested lies in *E* rather than *J*: both lie within the sequence equated within gene *D*. By determining the sequence surrounding the mutant amber codon, it is possible to define the phase in which gene *E* is read. This proved to be displaced one base to the right (that is in the direction of reading) from that of gene *D*. By scrutinising the sequence of gene *D* for an initiation codon (in association with a ribosome binding sequence) and a termination codon in this reading frame, it is possible to deduce that gene *E* resides within the latter part of gene *D*, and is read in a different phase (corresponding to a protein of 10,000 daltons, less than some previous estimates). Thus this sequence of DNA codes for two proteins.

How this situation may have evolved can be a subject only of speculation, but Barrell *et al.* suggest that probably gene *D* arose first, and that some event then led to the translation of part of it in a different phase, it so happening that the protein product possessed a high proportion of hydrophobic amino

acids that rendered it suitable to interact with the cell membrane, leading to the evolution of a lysis function. Of course, the evolutionary constraints upon the sequence representing both proteins must be very severe and it remains difficult to estimate how much scope there might be for variation in the sequences of the two overlapping functions. And, of course, overlapping expression must mean that all mutants in gene *E* also carry alterations in gene *D* (and *vice versa* within the region of overlap): while some of these mutations may affect one protein but not the other (due to third base degeneracy) it will be interesting in evolutionary terms to determine the general relationship between effects on *D* and *E* function. A possible utilisation of the overlap by the phage as a control is that one might expect ribosomes translating *D* to interfere with access to the initiation codon of *E*; this predicts that mutants causing termination early in *D* might have increased expression of *E*.

The complete sequence of the phage DNA will no doubt reveal whether the strategem of overlapping expression is used elsewhere in it, but the possibility is at least raised by the observation that the products of genes *A*, *B* and *C* have an estimated total molecular weight apparently somewhat greater than could be coded by this region of the genome.

What is the organisation of the genome of the filamentous phages? The genetic map constructed by Lyons and Zinder (*Virology*, **49**, 45; 1972) places the eight genes in a circular array; and with one reversal of order this was confirmed by the restriction maps calibrated with mutant locations in several laboratories (Van den Hondel *et al.*, *Eur. J. Biochem.*, **53**, 559; 1975; Seeburg and Schaller, *J. molec. Biol.*, **92**, 261; 1975; Horiuchi, Vovis and Zinder, *J. molec. Biol.*, **95**, 147; 1975). Proteins have been identified for six of the genes in two series of experiments; the results are in good agreement and suggest a total for the phage protein products of about 206,000 daltons (Model and Zinder, *J. molec. Biol.*, **83**, 231; 1974; Konings, Hulsebos and Van den Hondel, *J. Virol.*, **15**, 570; 1975). In addition, Model and Zinder identified a protein of 12,000 daltons, apparently phage coded but not yet equated with any gene.

Varying estimates have been taken for the size of the filamentous phage genome. Taking an estimate of 6,400 bases, and assuming that the genes *VI* and *VII* whose products are unidentified are about 250 bases each, led Van den Hondel *et al.* (*op. cit.*) to infer the presence of an unaccounted region of some 300 bases, which they located between genes *II* and *V* and suggested

might code for the protein of 12,000 daltons. Taking an estimate of 6,800 bases, and assuming that genes *VI* and *VII* are small, led Vovis, Horiuchi and Zinder (*J. Virol.*, **16**, 674; 1975) to suggest that there must be a considerable amount of intercistronic material, which they placed between genes *IV* and *II*. The assumption that genes *VI* and *VII* are small is supported by the mapping of known mutations to restriction fragments, but as is clear from the location of unaccounted regions in different sites, there is some latitude in the construction of the map, resulting principally from the uncertainty as to where the ends of genes lie. (The restriction fragments of these phage genomes have not been sized with respect to outside standards but only relative to each other, which makes the construction of a physical map dependent upon the size assumed for the total genome.)

It has generally been assumed that the size of the filamentous phage genome is close to 15% greater than

that of ϕ X. This would suggest a length of not more than 6,200 bases, which is only 600 bases greater than the 5,600 bases required to code for the six proteins of identified origin. If there were no intercistronic region, this would leave genes *VI* and *VII* able between them to code for some 22,000 daltons of protein. If an unidentified gene codes for the 12,000 dalton protein, then only 300 bases (or 11,000 daltons of coding capacity) remains for both these genes. In contrast to these calculations, Berkowitz and Day (*J. molec. Biol.*, **102**, 531; 1976) used biophysical means to measure a length for fd DNA of 5,740 (± 210) nucleotides. This would at best place a severe restriction on the length of DNA available to represent genes *VI* and *VII*; and if these genes should prove to code for larger protein products, or if the 12,000 dalton protein proves to represent a new gene, there would be the same paradox as seen with ϕ X174, with insufficient DNA to code for the phage products. \square

Lymphoid cell interactions in Japan

from Marc Feldmann

A Symposium entitled Cell Interactions in the Initiation and Regulation of the Immune Response, organised by Drs Yamamura and Hamashima, and sponsored by the International Union of Immunological Societies, was held in Kyoto, on September 1-4, 1976.

CELL interactions in the immune response were first recognised in 1966, with the work of Claman and his colleagues¹, and rapidly confirmed and extended by Miller², Davies³, Mitchison⁴, and others. At that time only interaction (or collaboration) between thymus derived (T) and bone marrow derived (B) lymphocytes was known, but the evidence of the following 10 years has shown that every immunological phenomenon involves interactions between several distinct cell types and their products. This symposium covered the whole spectrum of

modern cellular immunology and only a few of the aspects discussed can be reported here. Since lymphocyte heterogeneity is at the root of all these interactions recent developments in this field will take priority.

The many different surface antigens of lymphocytes have been exploited to purify different lymphocyte populations^{5,6}. The Thy-1 antigen which distinguished T from B cells⁶ is the most used. The three best characterised Ly (lymphocyte) alloantigens, Ly-1, Ly-2, and Ly-3, have been used by Cantor, Boyse and colleagues to define three subsets of T cells, bearing Ly-1,2,3, Ly-1, or Ly-2,3 antigens, which are present in decreasing proportions in the peripheral lymphoid pool.

H. Cantor (Harvard University) summarised recent work showing that helper T cells and cells involved in the mixed lymphocyte reaction (Ly-1) are distinct from cytotoxic T cells (Ly-2,3)⁷. The fact that suppressor T cells are also in the Ly-2,3 subset⁸, which is the smallest (5-10% of peripheral T cells) raised the interesting possibility that suppressor cells and cytotoxic cells may be identical, and the specificity of allo-aggressive suppressor cells like killer cells for the H-2K or D regions, rather than the I region was cited to support this view⁹. However, recent studies by Beverley *et al.*¹⁰ indicate that antigen specific suppressor cells (reactive to proteins) may be separated from cyto-

Correction

E. G. RICHARDS in an article in *News and Views* (*Nature*, **263**, 369; 1976) on September 30 entitled 'Complementary mispairs' inadvertently misnamed transitions and transversions on page 369. The word "transversions" on line 21 should be replaced by "transitions" and *vice versa* on line 24.

toxic T cells (reactive to H-2 antigens) by the use of anti-Ia antiserum, which lyses the former, and also by anti-Ly-1.1, which lyses cytotoxic cells of the CBA strain. On the other hand, the precursors of suppressor cells are usually not lysed by anti-Ia antiserum¹¹, and thus the two cell types could still be closely related.

It is now clear, however, that the Ly-1 and Ly-2 populations belong to different lines of differentiation and are not sequential stages in a single lineage¹⁴. Cantor and his colleagues have shown that mice depleted of T cells (thymectomised, irradiated and bone-marrow-reconstituted mice or "B mice") and repopulated with Ly-1 T cells only were not able to perform any of the functions of Ly-2,3 cells, even many months later; and conversely "B mice" repopulated with Ly-2,3 cells could express cytotoxicity and suppression, but not other T cell functions.

There is great interest in the role of the major histocompatibility complex (MHC) in immune responses. One aspect which has attracted attention is the prohibition of cell interactions across allogeneic barriers where MHC differences are involved. This has been reported for T-B interactions by Katz and colleagues¹⁵, for T-macrophage interactions by Rosenthal and Shevach¹⁴ and Erb and Feldmann¹⁶, and for T cell killing, by Zinkernagel, Blanden and Doherty¹⁷. Several explanations have been suggested for these restrictions. One is that a molecule controlled by the MHC must be "shared" or recognised for successful interactions to occur¹⁵. An alternative suggestion is that mixing histoincompatible cells may induce active suppression^{18,17}. D. H. Katz (Harvard University), for example, found that suppressor cells are induced when F₁ thymocytes are primed in an irradiated parental mouse. These suppressor cells may explain the genetic restrictions on T-B collaboration found in earlier experiments¹⁵. Support for this possibility was discussed by H. Cantor and R. K. Gershon (Yale University) who have shown that removal of Ly-2,3 cell populations (which contain suppressor cells) permits the residual T cells to collaborate with histoincompatible B cells in the primary response *in vitro* to sheep red cells. Independently, Dutton and his colleagues¹⁷ have obtained similar results using anti-Ly antisera and cell separation techniques, indicating that allogeneic effects can suppress interactions between histoincompatible T and B cells. Thus, there is no doubt that suppression explains some examples of the failure of histoincompatible T and B cells to interact. Whether all such failures can be attributed to suppression is not yet clear.

EGF and viral transformation

from Robin A. Weiss

EPIDERMAL growth factor (EGF) was discovered by Stanley Cohen (*J. biol. Chem.*, **237**, 1555; 1962) when he was purifying nerve growth factor from the submaxillary salivary gland of male mice. Cohen noted that the factor stimulated the proliferation and differentiation of the epidermis, as reflected by precocious opening of the eyelids and eruption of the incisors in baby mice. Since that time it has been Cohen's remarkable achievement to purify and sequence murine EGF (Cohen and Savage, *Recent Progress in Hormone Research*, **30**, 551; 1974). It is a single-chain polypeptide containing 53 amino-acid residues with a molecular weight of 6,045. The molecule has been well conserved during evolution and human EGF extracted from urine shows only minor differences in amino-acid sequence to murine EGF. Recently it has become evident that EGF will stimulate proliferation of a variety of both epithelial and fibroblastic cells in culture. Its specific mitogenic action on non-proliferating cells in culture provides a much more convenient and quantitative assay than examining eyelid opening *in vivo*.

Last year Cohen's group showed that cells in culture which respond to the mitogenic action of EGF possess specific receptors for EGF on the cell surface (Carpenter, Lembach, Morrison and Cohen, *J. biol. Chem.*, **250**, 4297; 1975). EGF receptors were assayed by measuring the binding of ¹²⁵I-labelled EGF to the cell surface. Cells of many species bound EGF, but certain types, including a human lymphoblastoid line (NC-37) and rat cells transformed by mouse sarcoma virus

(KNRK) and by Rous sarcoma virus (XC) apparently lacked EGF receptors. Reduction in the binding of other hormones to transformed cells has also been reported, for example, in a recent study of insulin receptors on BALB/3T3 cells by Thomopoulos, Roth, Lovelace and Pastan (*Cell*, **8**, 417; 1976). The binding of insulin varied with the level of proliferation in cell population and the reduction seen in transformed clones was not specific to the transforming agent. However, the presence of EGF receptors appears to be more specific. In this issue of *Nature* (page 26), Cohen, in collaboration with Todaro and De Larco, reports additional data on the binding of EGF to the same cell types transformed by different tumour viruses. For example, the A31 clone of mouse BALB/3T3 cells was transformed by SV40, two strains of mouse sarcoma virus (MSV) and Rous sarcoma virus (RSV). Only the MSV-transformed subclones failed to bind EGF. This remarkable specificity of binding was true for transformed subclones of other types of cell such as Swiss 3T3, NRK and Mink lung. In each case, only the subclones transformed by MSV or by feline sarcoma virus failed to bind EGF.

Solely on the basis of the specific failure to bind EGF, the authors postulate that MSV might transform cells by coding for an EGF-like molecule in part of its sarcoma-specific genetic sequences. This EGF analogue would then block the receptors and stimulate the cells to grow. This will hardly provide the whole explanation of viral oncogenesis, but it can be tested.

It now seems clear from the reports of several workers that suppression is mediated by products of the I region. T cell suppression in mice, for example, is mediated by an antigen-specific T cell factor with a molecular weight of about 50,000 and which does not operate across H-2 barriers^{18,19} (T. Tada, Chiba University). This factor is absorbed by antisera reactive to products of the I-J subregion, which also seems to code for the receptor for the suppressive factor. The cells carrying the receptors (sometimes known in this context as acceptors) were found not to be helper T cells, but nylon-wool-adherent T cells which could be killed by anti-I-J serum and complement^{18,19}. Allotype specific suppressor T cells can also be killed by antiserum containing antibody to I-J region products²⁰⁻²² (L. Herzenberg

and D. B. Murphy, Stanford University).

Two generalisations seem to be possible on the basis of this and other work. First, that the I region is functionally specialised, the I-J subregion being involved in suppression and the I-A subregion in macrophage or T-cell helper functions. Second, the products of the I region act on target cells through receptors controlled by the same I subregion^{23,24}.

M. Taniguchi (Chiba University) discussed studies performed with Tada on a factor obtained from lysed antigen-primed spleen or thymus cells which augmented IgG antibody responses, provided that factor donor and recipient (in culture) were identical in the I-A region. This factor seems to be different from the helper factor described by

Taussig and Munro²² which does not require I-A identity. Furthermore, the material isolated by Taniguchi and Tada unlike that of Munro and Taussig does not induce immune responses in the absence of other T cells, suggesting that it is an amplifier factor, rather than a helper factor which replaces T cell function. Thus, there are now four examples of I region factors each of which acts through a receptor on another cell type, but which is controlled by the same I subregion¹⁸⁻²⁴. The presence of (at least) three pairs of genes for factors which augment responses and their receptor each detected by different techniques in different assay systems, but all controlled by I-A, leads to the obvious question as to whether the factors described by Taussig²³, Erb²⁴, and Taniguchi¹⁸, are really distinct members of a 'family' of factors, or whether they are the same, but seem different because of the assays used. T cell suppression is also under control of non-H-2 genes. This was reported by Tada on the basis of the inability of cells from the A/J mouse strain to make suppressor factor, and of cells of the B10 congenic strains to accept suppressor factor^{18,19}.

The mechanism of suppression of the immune response by T cells is not yet known in detail. Tada noted that suppression involves an intermediate cell, which is involved in suppressing helper cells. J. F. A. P. Miller (Walter and Eliza Hall Institute, Melbourne) reported that suppressor cells induced during tolerance induction cannot suppress on their own but require the participation of adherent cells²⁵. E. Diener (University of Alberta, Edmonton) also described studies in which adherent cells were involved in the mediation of tolerance²⁷. This suggests that adherent cells (perhaps T cells, or cells of the macrophage/monocyte series) are involved in the effector phase of suppression. Clearly the relationship of this cell, which may be of hitherto unknown type, to known cell types, is an important question for classification.

Miller's work emphasised that we do not yet know how many types of lymphocyte there are. He reported that cells which suppress induction of the delayed hypersensitivity response in mice are Ly-1 cells, unlike the Ly-2,3 suppressor cells which inhibit antibody response. This difference could be explained if there is T-T interaction in induction of the suppressor cells of the delayed hypersensitivity response with Ly-1 cells stimulating (*in vivo*) Ly-2,3 precursors of suppressor cells to become active suppressor cells. Such a T-T interaction in the induction of suppressor cells has been reported previously²⁸. T. Hamaoka (Osaka Uni-

versity, Japan) reported studies which confirmed the T-T interaction concept, using hapten reactive T cells.

The field of cell interactions in the immune response has clearly expanded considerably over the past 10 years, and it is predictable that the next 10 years will see a deepening awareness of the complexity and richness of the cell interactions and suppressive effects which regulate and integrate immune response.

- 1 Claman, H. N., Chaperon, E. A., and Triplett, R. F., *Transplant Rev.*, **1**, 92-113 (1969).
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Gravity experiments

from Malcolm MacCallum

STANDARD introductory courses in general relativity give an account of the "three crucial tests" discussed by Einstein in his famous 1916 paper: the gravitational redshift, the bending of light-rays and the advance of perihelia of planets, especially Mercury. These established the superiority of Einstein's theory to the others of the day, but many more theories have been invented in the intervening 60 yr. Only since 1970 have new experimental tests become available.

The classic tests are of course still actively pursued. The Eötvös experiment, which checks that the gravitational acceleration of laboratory bodies is independent of their composition, was improved to one part in 10^{13} by

V. A. Braginsky and V. I. Panov in 1971. Gravitational redshift measurements, both astronomical and terrestrial, which can be interpreted as a test of the universality of free-fall (given energy conservation), are accurate to a few per cent. Clocks in spacecraft should improve on this, and a satellite experiment is already in progress.

It is difficult to carry out the second "crucial test" with visible light. The Texas team which recently reported on observations made in Mauretania in 1973 (*Astr. J.*, **81**, 452; 1976) quote an error of about 12%. Measurements of the deflection of radio waves are more accurate. The most recent result, announced by E. B. Fomalont and R. A. Sramek (*Phys. Rev. Lett.*, **36**, 1475; 1976) quotes an error of about 1%. This limit cannot really be improved without detailed knowledge of the conditions in the solar wind plasma through which the waves pass.

The perihelion measurements have been checked by radar to about 1%. Their meaning was thrown into question 9 yr ago when R. H. Dicke and H. M. Goldenberg observed an apparent oblateness of the Sun which suggested a solar quadrupole moment large enough to produce a significant part of the effect, but H. A. Hill and R. T. Stebbins (*Astrophys. J.*, **200**, 271; 1975) found a much smaller corrected value. All three of the "crucial tests" are thus in good agreement with Einstein's theory.

It has been known for some time that further effects are potentially observable. The first of these actually to be measured was the "fourth test of general relativity", the time-delay of electromagnetic waves passing near the Sun. This has been measured by radar echoes from Mercury and Venus and more recently, by J. D. Anderson *et al.* (*Astrophys. J.*, **200**, 221; 1975), using the Mariner 6 and 7 spacecraft, to about 3%.

Possible experiments on gravity have been extensively examined since 1968 by K. D. Nordtvedt at Montana State University and by a group at California Institute of Technology led by K. S. Thorne. The simplest theories to analyse are those in which the influence of gravity on matter is completely described by a (Riemannian) metric, as it is in general relativity. These theories differ in how the matter generates the metric. The analysis proceeds by computing all possible effects arising from the lowest order corrections to Newtonian theory. The most general form of this "parametrised post-Newtonian framework", due to C. M. Will (*Astrophys. J.*, **185**, 31, 1973) involves 10 parameters dependent on the particular theory considered. Theories which do not fit within this framework are more difficult to analyse systematically, but several of them have already been

shown to be in conflict with observation. The programme has drawn attention to the possibility of new types of experiment and new experimental results on two of these have been announced recently.

One of them is the Nordtvedt effect, which arises if gravitational energy does not obey the equivalence of inertial and passive gravitational mass. Since the gravitational energy content of laboratory bodies is extremely small, this is not tested by the Eötvös experiments. Laser-ranging of the Moon has been carried out since 1969 to search for the effect. The results obtained and analysed by a team of seventeen from nine American institutions (J. G. Williams *et al.*, *Phys. Rev. Lett.*, **36**, 551; 1976) and independently analysed by I. I. Shapiro, C. C. Counselman III and R. W. King (*Phys. Rev. Lett.*, **36**, 555; 1976) show that the equivalence principle for gravitational energy is true to about 3% accuracy.

The other new result concerns variations in gravimeter readings, like those produced by Newtonian tides of the solid Earth. As shown by C. M. Will, these variations could contain post-Newtonian contributions dependent on lunar, solar and sidereal periods with 12-h, 24-h and annual oscillation. Most of them are too small to be detectable. Nevertheless, existing data enabled Will to place strong limits on the influence of any preferred reference frame (essentially an absolute rest frame), and to show, for example, that Whitehead's action-at-a-distance theory of gravity, which had lasted 50 yr, predicted effects 200 times larger than experiment allowed. Effects of these types have been further restricted by new data produced by R. J. Warburton and J. M. Goodkind of the University of California at San Diego (*Astrophys. J.*, **208**, 881; 1976) using a superconducting gravimeter. The main uncertainty lies in the calculation of the Newtonian effects, especially those arising from the shifting weight of the oceans.

All the experiments mentioned above, together with those also analysed by Will on the equivalence of active and passive gravitational mass and on the Earth's rotation rate, are in agreement with the predictions of general relativity. The only discrepant result is the time-dependence of the gravitational constant given by T. C. van Flandern (*Mon. Not. R. ast. Soc.*, **170**, 333, 1975) but even this differs from zero only by twice the quoted error. Further results from these measurements and from, for example, the precession of gyroscopes in satellites, can be hoped for in the next decade.

One might thus think that Einstein's theory was proved. Certainly the work

of the past 5 yr has severely damaged the credibility of those rival theories available in 1970. Unfortunately, any finite number of effects can be fitted by a sufficiently complicated theory. Indeed, all the general-relativistic predictions for the post-Newtonian effects can be replicated to arbitrary accuracy or even exactly by suitably constructed alternative theories. Aesthetic or philosophical motives will therefore continue to play a part in the widespread faith in Einstein's theory, even if all tests verify its predictions. □

Histone gene organisation

by Eleanor Lawrence

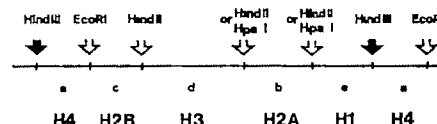
OVER the past few months a detailed picture of histone gene organisation at the level of the repeating unit has begun to emerge, based on the work of several groups who have approached the problem with a variety of different techniques.

In a recent set of papers (Gross, Probst, Schaffner and Birnstiel, *Cell*, **8**, 455; 1976; Schaffner, Gross, Telford, and Birnstiel, *Cell*, **8**, 471; 1976; Gross, Schaffner, Telford and Birnstiel, *Cell*, **8**, 479; 1976) Max Birnstiel and his colleagues have ordered the individual genes within the histone repeat unit of sea urchin DNA—in this case *Psammechinus miliaris*. They have ordered the genes by hybridising restriction endonuclease-generated fragments of enriched *Psammechinus* histone DNA with highly-purified individual labelled histone mRNAs isolated from the labelled 9S polyribosomal RNA from a closely related sea urchin *Paracentrotus lividus*, each of which has been assigned to one of the five histones H1, H2A, H2B, H3 and H4, by comparison of the *in vitro* translation products with *Paracentrotus* histones labelled *in vivo*.

A major difficulty in previous attempts to order the individual coding sequences in this way, has been the separation and identification of mRNAs coding for histones H2A, H2B and H3. By a careful choice of conditions, based on a study of the complex behaviour of *Paracentrotus* histone mRNA on electrophoresis under various denaturing conditions, Gross *et al.* were able to obtain five distinct mRNAs of sufficient purity that on translation in a wheat germ cell-free system four of the mRNAs could be characterised as coding mainly for a single protein which could then be identified as one of the histones by comparison with ³H-leucine and ³H-lysine labelled histones produced *in vivo*.

From digestion of enriched histone

DNA by a set of four restriction enzymes a detailed restriction map of the complete unit was constructed. Using combinations of restriction enzymes the 6 kb complete repeat unit was subdivided into five fragments. Each fragment hybridised with only one of the purified mRNA fractions and so by collating the restriction map and the hybridisation data, the individual coding sequences could be ordered within the repeat unit as shown below.



The polarity of the *Psammechinus* histone genes has also been determined. Limited resection by lambda exonuclease (which digests from the 5' end only) of the complete repeat units obtained after restriction with *HindIII* or *EcoRI*, and subsequent hybridisation to H4 mRNA showed that the polarity of the H4 gene was 5' H2B→H4→H1 3'.

Further experiments of this and other groups have shown that the histone coding sequences all lie on the same DNA strand, and so it follows that all the coding sequences have the same polarity, and transcription will proceed in the direction H4→H2B→H3→H2A→H1.

Groups at Stanford and CalTech have since reported a similar order for the histone genes in another sea urchin—*Strongylocentrotus purpuratus* (Cohn, Lowry and Kedes, *Cell*, **9**, 147; 1976). In this case pure histone DNA was isolated by cloning the two *EcoRI* restriction fragments of ~2.2 and ~4.8 kb, which make up the complete '7 kb' histone repeat unit in plasmids pSp17 and pSp2, respectively. Further dissection with restriction endonucleases enabled a detailed restriction map of the repeat unit to be constructed and fragments corresponding to various classes of histone mRNA were identified by hybridisation. An earlier tentative assignment of these mRNAs to individual histones (see *News and Views*, **259**, 361; 1976) has required revision, and the order of the genes has now been determined by direct sequencing of portions of the plasmid-cloned DNA fragments corresponding to the different mRNA classes (Sures, Maxam, Cohn, and Kedes, unpublished) and matching them with the known histone amino acid sequences. The genes appear to be ordered in exactly the same sequence as those in *Psammechinus*.

A detailed quantitative assessment of the relative positions of the coding and spacer regions is provided in the

accompanying paper by Wu *et al.* (Wu, Holmes, Davidson, Cohn and Kedes, *Cell*, **9**, 163; 1976), who have hybridised total histone mRNA to single-stranded DNA from the histone plasmids pSp2 and pSp17.

Since the mRNAs hybridise only to the complementary coding sequences, the arrangement of coding and non-coding regions can be seen directly in the electron microscope after a novel treatment with T4 gene 32 protein which preferentially stains the single-stranded regions of the histone plasmid DNA-histone mRNA hybrids (Wu and Davidson, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 4506; 1976). As expected, they found three RNA:DNA hybrid regions on pSp2 and two on pSp17 accounting for the five histone genes, interspersed with spacer DNA—definitive proof that each gene is only repeated once in each repeat unit. Direct measurements allow them to independently assign most of the duplex regions to specific histone genes, constructing a map which agrees with those obtained by other methods.

Portmann, Schaffner and Birnstiel

(page 31 of this issue of *Nature*) have also directly demonstrated by electron microscopy five coding regions interspersed with spacer DNA in the *Psammechinus* repeat unit DNA cloned in phage lambda, by making use of the fact that the AT-rich DNA of the spacer regions denatures at a lower temperature than the GC-rich coding sequences forming open loops along the DNA. The GC-rich regions correspond well in size to the individual histone protein sequences.

Detailed sequencing of the repeat unit at the nucleotide level is now underway, made possible by the ability to obtain pure DNA fragments by cloning in phage or plasmids. Before too long, nucleotide sequencing may well have identified any regions of homology between the non-transcribed DNA in these and other genes, or between the histone spacer sequences themselves. Preliminary evidence (Cohn *et al.*, *Cell*, *op. cit.*), indicates that most of the histone spacer sequences are unlikely to be shared with other genes, and that there is also little homology between the individual spacer sequences. □

keeping population levels steady

Hogarth concludes with speculation on the causes of extinction of dragons, despite persistent accounts of dragons and similar animals even in the present century, the typical mediaeval dragon was certainly extinct by the late 18th century. One contributing factor was commercial over-exploitation, primarily for pharmacological purposes. Only once was conservation legislation passed to protect dragons. This was in Rhodes, in 1345, when the king forbade any knight to attempt to slay a local dragon (although Hogarth conjectures that this edict stemmed from concern for the knights, not the dragon). If we accept the notion that dragons were extreme K-selected animals, then their rapid extinction under the diverse pressures exerted by man is not surprising (see for example, *Nature*, **257**, 737–738; 1975).

Hogarth's article is undoubtedly seminal, but I find it in some respects excessively uncritical. In discussing the evolution of dragons, and other "related species such as the cockatrice and griffon", Hogarth suggests they "probably originated as a distinct group only 5,000 years ago". Quite apart from the inherent implausibility of this statement, it is well to begin by getting clear the morphological details of the animals loosely grouped together here. These can be obtained from bestiaries, or from any heraldry text. Setting aside relatively minor differences, such as whether the feet have talons or claws, or whether the head has teeth or a beak, the basic difference is that the griffon and the canonical dragon are six-limbed (four legs, two wings), whereas the wyvern and cockatrice are four-limbed (two legs, two wings).

This is an absolutely fundamental distinction. One of the most conservative features of vertebrate evolution is the tetrapod morphology: this may be seen in any museum exhibit of the

The ecology of dragons

from Robert M. May

ALTHOUGH much studied in earlier times, dragons and their ilk have been largely neglected in the recent upsurge of interest in animal ecology and behaviour. An article by Hogarth (*Bull. Brit. ecol. Soc.*, **7**[2], 2–5; 1976) seeks to remedy this neglect.

In view of the lack of contemporary observational evidence, Hogarth necessarily relies on a survey of earlier sources. Most of these are from the 17th and early 18th century, an age when scientific curiosity was flowering. Later publications are increasingly sceptical, although Hogarth notes published doubts on the existence of dragons as early as Caxton's (1481) *Mirror of the World*.

Dragons appear to have been both omnivorous and voracious. Different records testify to their diet having been highly variable in both composition and quality: one dragon ate two sheep every day, and another which was kept captive by Pope St Sylvester consumed 6,000 people daily. The population density was also highly variable (presumably in a way which correlated with the per capita food requirements): "in England, indigenous dragons were solitary and it is doubtful whether the resident population averaged more than a few dozen, although occasional migrant flocks of up to 400 were seen: in India, by contrast, the marshes and mountains were described as being 'full' of dragons". Estimates of their life table parameters are scrappy, but

there seems to be general agreement on a typical lifespan of the order of 10^2 – 10^4 years.

The sexual display behaviour of dragons includes at least one remarkable and unparalleled manifestation, recorded by an 18th century author "Dragons, being incited to lust through the Heat of the Season, did frequently, as they flew through the Air, Spermatise in the Wells and Fountains". This may be conjectured to have had adaptive value in reducing intrinsic fecundity. Such long-lived beasts would seem to have been at the extreme K-selected end of the r-K continuum, and would therefore be likely to exhibit behaviour which had the effect of



Mary Evans Picture Library

500,000,000 years of evolution from lobe-finned fishes through amphibians and reptiles to birds and mammals. This underlying conservatism in skeletal structure, despite great variation in outward form and function, probably reflects the relative ease of modification of genes which govern timing in development, as opposed to those governing basic structure (see for example, King and Wilson, *Science*, **188**, 107-116; 1975). The wyvern and cockatrice have this basic vertebrate tetrapod morphology, but the six-limbed dragon and griffon do not. The probable ancestry of these latter two, as an entirely separate group, therefore dates back at least to the Devonian. This basic distinction applies to other now-extinct beasts despite superficial similarities, unicorns belong with the familiar tetrapods, but the pegasus belongs with the six-limbed dragon-griffon vertebrate phylum, as do centaurs. Some angels (the humanoid-plus-wings kind) also belong in this phylum, but in view of the bewildering complications of angel morphology (once one includes cherubim, seraphim, and so on; see Davidson, *Dictionary of Angels: Including the Fallen Angels*, Free Press, 1967), this point is best not pursued.

In brief, wyvern and cockatrice can be envisaged as radiations from the basic vertebrate theme. But dragons, griffons, centaurs and angels belong to an entirely different lineage, the evolutionary history of which is shrouded in mystery.

The loose association of these two fundamentally different groups provides a striking example of the pre-Darwinian tendency to regard each species as a separate act of creation, rather than to trace logical phylogenetic relationships.

On the other hand, grouping together dragons, wyverns and the like is understandable in the light of the similarities of their ecology, behaviour and superficial appearance. They provide a dramatic example of evolutionary convergence, in the face of phylogenetic differences at least 400,000,000 years old. Such convergence implies some very tight evolutionary constraint somewhere in the "dragon" niche, a constraint hardly hinted at in Hogarth's account of their highly generalist diet and behaviour. This constraint may lie in the tendency exhibited by most dragons of record to be obsessive custodians of hordes of gold.

I conclude with the time-worn call for further research, modified by the highly contemporary remark that (if the above speculation is correct) such research may yield the literally golden fruits that grant-giving agencies increasingly desire. □

Primate behaviour and ecology

The Sixth Congress of the International Primatological Society was held at Cambridge on August 23-27, 1976. Aspects of the conference dealing with behaviour and ecology are discussed below

from F. P. G. Aldrich-Blake and
Miranda Robertson

THE theoretical thrust of much primate work in the late sixties and early seventies was loosely socio-ecological; features of social organisation such as group size and composition were related to broad ecological categories of habitat such as 'forest' or 'savanna'. While this approach sought an evolutionary explanation of primate societies, it was insufficiently precise both in its treatment of causal mechanisms of social change and in its measurement of critical ecological variables. Recent work has placed a greater emphasis on the adaptive strategies of individuals in their dealings with society and the environment.

Notable in this respect was a paper by R. M. Seyfarth, D. L. Cheney and R. A. Hinde (University of Cambridge), which sought to provide a conceptual framework within which to interpret inter-individual behaviour. Primate societies, they pointed out, can be analysed at three different levels: interactions between individuals; the long-term relations to which interactions give rise; and the structure resulting from those relationships. While patterns of interaction between individuals are often apparently complex, they may be governed by relatively simple principles. For example, networks of social grooming among adult females of four species of primate had many features in common, despite being drawn from groups of differing size and degree of genetic relatedness. Computer simulation showed that these features could be accounted for by a preference for females of high rank as grooming partners and competition for access to these preferred females. Interactions between individuals have long-term effects on their relationships; grooming partners are more likely to form coalitions during aggressive encounters than are pairs that have had fewer friendly interactions in the past. Thus the optimum individual strategy should be to maximise the benefit derived from others by maximising the time spent interacting with animals of high rank. A similar theoretical approach can be used to explain many other aspects of social behaviour; the different behaviour of immature males and females, for instance, can be related to

strategies for maximising their fitness as adults.

In contrast, sessions on ecology produced few conceptual syntheses, despite a striking increase in the quality and quantity of research in this field. Detailed accounts of primate communities in Asia, Africa and the Americas were presented, the most notable being that of T. T. Struhsaker (New York Zoological Society) and his associates in the Kibale Forest, Uganda. In all areas, leaf eaters attained higher biomasses than fruit eaters, and these higher than insectivores, but otherwise few general principles emerged. Indeed discussion revealed dissent on aims and methods, let alone conclusions.

We still do not know what factors limit the population of any primate. Availability of food is clearly a plausible candidate; K. Milton's (New York) studies of the howler monkeys of Barro Colorado Island and their habitat suggest that fruit and young leaves of suitable quality may be in short supply at some seasons. Food intake may be limited as much by the need to avoid toxins as to obtain nutrients. Milton showed that many potential foods were rich in phenolic compounds, and J. S. Gartlan and D. B. McKey (University of Wisconsin) likewise demonstrated the presence of toxins in most plant products in the Douala-Edea forest of Cameroon. On the other hand T. Iwamoto (Miyazaki University), in bioenergetic studies of Japanese monkeys in evergreen forest and gelada baboons in Ethiopian montane grassland, obtained figures suggesting that primates used only a tiny proportion of the potential food available. T. H. Clutton-Brock (University of Sussex), and other participants in the concluding discussion, considered that problems of measuring food availability were so intractable, at least in tropical forest, that any attempts were doomed to failure.

S. A. Altmann (University of Chicago) suggested that attention should be focused on species living in relatively simple habitats, and presented a mathematical model of optimal diet, to be tested on savanna baboons in Kenya. Foraging strategies to be adaptive, he pointed out, animals must eat enough of the various foods available to stay above the minimum for every nutrient and below the maximum for every toxin, at the least possible cost. While the model related these factors with elegance and simplicity, many participants thought it likely to founder on the practical problems of measuring cost, including as it does such diverse elements as energy expenditure, time, and risk of predation. Clearly we must wait some years yet before the value of this and competing approaches becomes clear.

One aspect of research on primate social organisation which seems to be relatively underdeveloped is that of communication, which was the subject of a review lecture by Peter Marler (Rockefeller University). It is still not known for certain whether the dozen or so sounds that make up the communicative repertoire of most monkeys and apes are ever used in the way that words are used to make sentences—one sound retaining the same meaning in different sound combinations—or whether it is always the complete pattern that conveys the message, and not the sum of its parts. It is almost certainly the entire sound combination, but the precise interpretation of primate calls in their natural context can be extremely difficult and Marler took the view that the effects of playing back natural calls should be very illuminating, perhaps especially in the laboratory in combination with single-unit recordings from the animals' cortex. (Neurophysiologists, who have had more experience with the problems of interpreting the calls of single units in their natural surroundings, might take issue with him on the latter point.)

What apes have certainly not done is to develop a language in the sense that man has, and in spite of the research resources poured into the attempt, they have not been conclusively shown to be able to use ours in a strictly linguistic way. Although D. M. Rumbaugh (Yerkes Primate Center, Atlanta) protests the contrary, David Premack (University of Pennsylvania) not only conceded that apes never have grasped human language, but is willing to predict that they never will.

They can, however, fairly readily learn to use abstract symbols to represent objects and actions, and they can use the symbols in strings. What is missing is the evidence that the strings are composed according to syntactic rules.

Tired of trying to satisfy professional linguists that ape use of symbols fulfils the criteria for linguistic competence, some researchers have declared they would be satisfied by a "Turing test". If a man cannot tell whether he is conversing with an ape or another man, then the ape is using language. P. A. M. Seuren, collaborating with G. Ettlinger and others at the Institute of Psychiatry (London University), has, as a linguist himself, suggested a more sophisticated test which hinges on an anomaly of human usage. We say, for instance, "Don't do this and don't do that" or "Don't do this or that", but never the logically equivalent "don't do this and that". That linguistic quirk, according to Seuren, is virtually universal in human languages. The question is, would it crop up spontaneously

in ape usage? The answer will not be available until Ettlinger and his colleagues have succeeded in teaching apes to use "or", which has so far not been done. □

Heavy ions at Caen

from P. E. Hodgson

The European Physical Society Conference on Nuclear Physics with Heavy Ions was held at Caen on September 6–10, 1976

HEAVY ion physics has developed rapidly over the last few years and the conference provided an opportunity to survey the areas that are already quite well understood, and also to discuss the extensive new data on new phenomena, and the theories that have been suggested to account for them. Caen was chosen for the Conference because a powerful new heavy ion accelerator GANIL is to be built there in the next few years.

Glancing collisions between heavy ions, in which one or two ions are transferred, are useful probes of nuclear structure, and the present state of this work was surveyed by O. Hansen and J. Garrett (University of Copenhagen). Detailed theories of these reactions have been developed, and very complicated theoretical calculations, involving the solution of coupled Schrodinger equations, are needed to analyse the data. K. Low (CEN, Saclay) showed how these calculations account for the details of the reaction mechanisms. Their very complexity does, however, make it difficult to use such reactions for nuclear structure studies. This work also needs the optical potentials that represent the interaction between the ions, and some of the latest methods of calculating these potentials were surveyed by D. Brink (University of Oxford).

Extremely complicated reactions take place when the heavy ions collide more nearly head-on. They can fuse together to form a highly excited compound system which then decays by particle emission (discussed by M. Lefort (Orsay)). If the collision is intermediate between glancing and head-on the two nuclei can orbit round each other and exchange a few nucleons, becoming highly excited in the process, before separating again. Measurements have been made of the energies and masses of the ions after these deep inelastic interactions, and some of the results were considered by J. Galin (Orsay) and by L. G. Moretto (Berkeley). The theories that have been developed to account for these data were summarised in a comprehensive review by W. Norenberg (University

of Heidelberg). The earlier theories using the classical concept of friction are now being replaced by microscopic transport theories and the spreading of the energy distribution is described by the Fokker–Planck equation. This new and detailed understanding of deep inelastic scattering was one of the more important developments reported to the conference.

When two heavy ions combine after a non-central collision, the compound system has a large angular momentum. This preferential excitation of high spin states is a useful means of studying nuclear structure, and the development of our knowledge in this area was surveyed by Z. Szymanski (University of Warsaw).

A special session was devoted to the many attempts that have been made in the past few months in several laboratories to detect superheavy nuclei, after the announcement that evidence has been found for their natural occurrence in the mineral monazite (see *Nature*, 261, 627; 1976). Samples of monazite have been bombarded with protons and the spectra of the gamma rays subsequently emitted have been measured. Detailed chemical analyses have been made of samples of the mineral and reactions between heavy ions that might lead to their production have been studied. Monazite has been bombarded with heavy ions, and the recoils examined to see if any of them could have come from superheavy nuclei, but none of this work provided any evidence in support of their existence. This does not yet destroy the evidence offered in the original papers, as there are still several important points that require more detailed examination, but the evidence in favour of superheavy nuclei now looks decidedly weaker than it did a month ago. Work will certainly continue in this exciting field. □



A hundred years ago

REFERRING TO OUR recent correspondence on "Antedated Books," a correspondent calls attention to another evil practice that has of late years crept into the publishing trade, namely, that of publishing books without any date at all. Such books are generally of small literary or scientific value, but circulate among a class who are generally unable to test their value. Of course the purpose of issuing undated books is evident; works half a century old may be palmed off on the unknowing as the genuine product of the current year.

From *Nature*, 15, November 2, 1876.

review article

Development of early stock rearing in the Near East

S. Bökönyi*

The neolithic "revolution", from hunting and gathering in human communities to animal husbandry and agriculture, seems to have originated in the Near East. A primitive kind of domestication arising from the adoption of the young of wild beasts killed by hunters seems to have preceded the deliberate breeding of domesticated strains.

STUDIES on the domestication, origin and development of domestic animals have advanced particularly rapidly in the period since the Second World War. Among those areas to which archaeozoological research has extended essentially only in the past three decades, the Near East (South-west Asia with the southern part of Turkestan) seems to be the most interesting at the moment. There are several reasons. One is that the neolithic "revolution" or "transformation", whose essence was the switch from food gathering to food production, and whose one basic innovation was animal domestication, started in its most interesting and exciting form there. It is also important that the neolithic development of Europe was connected by numerous threads with the neolithic development of the Near East. From this viewpoint too it is especially important that both of the most important domestic species which determined the character of animal husbandry of the earliest Neolithic originated in the Near East.

Before discussing the problems of animal domestication in the Near East, it is useful to begin with a brief summary of the wild forms, their habitat and area of distribution.

The aurochs, the wild ancestor of cattle, spread over the whole Near East in the early post-Pleistocene period. Its remains occur in almost every neolithic sites of the area. The considerable size of these aurochs suggests that the geographical environment must have been favourable. When the plains and hills dried up and were reduced to steppes, the number of aurochs sharply diminished while small isolated populations developed which gradually died out. Around the time of Christ aurochs could be found only in inaccessible mountainous retreats. The typical habitat of the species was the forest steppe or the light forest. It was more an animal of the plains and hilly regions than the bison, its close relative, which was a good climber and preferred dense forests.

Wild sheep occur in abundance in the mountains of the whole Near East excluding the Levant. Their typical habitat is the mountains, though they also live on broad plateaux, climbing over 4,000 m above sea level in summer, and descending into the valleys in winter time.

The bezoar goat, the wild form of our domestic goats, lives all over the mountains from the Levant, through Anatolia, to the Hindu Kush. Its habitat is the high mountains with steep, rugged cliff facings and ravines.

The wild swine was widespread in the whole Near East, in marshy areas and near lakes and streams, in prehistoric times. It still lives in many regions there. It is an inhabitant of wet environments, preferring dense forest, but also living in the bush or among the bush or among the reeds along waterflows.

The wolf, the wild form of the domestic dog, is to be found all over the Near East and shows an extremely wide habitat tolerance that ranges from forest to desert and from mountains to plains.

The habitat preferences of the wild forms described above are a good key to determining in which environmental type of the Near East domestication took place. Steppe and desert can immediately be excluded, for the wolf was the only species which could live there (the dog had no important role in the prehistoric economy of the region). On the other hand, in mountains and their foothills all five species could be domesticated, and pig domestication could also take place in swampy areas of any geographical region.

The domestication of animals was by no means a sudden development, but was preceded by a man-animal relationship which went back hundreds and thousands of years. One must not forget that the relationship between the hunting man and the hunted animal was not simply the killing of the prey. Tens of thousands of hunter generations accumulated and passed on much information on the behaviour, anatomy, biology, physiology, and so on of wild animals, so that the domestication began on well prepared ground. Domestication was undoubtedly connected with a settled life, in common with other aspects of the neolithic revolution. Nevertheless, a settled life was not an indispensable precondition: domestication can happen (and in fact did happen) without settlements, more often however in extreme environmental conditions (for example, reindeer domestication of Arctic peoples), and in such cases real animal husbandry with a wide variety of domestic species never or rarely developed.

It is impossible to resolve the question of whether settled life made domestication possible or vice versa. Probably both are true: it is hardly conceivable that keeping and taming and subsequently breeding a larger number of captured wild animals could occur without corrals or stalls, necessitating at least temporary settled life. It is as inconceivable to supply food to a larger settled community without domestication and animal keeping or at least herding.

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The primary stimulus for animal domestication was probably the drastic climatic and environmental change which followed the end of the Pleistocene. In the Near East the end of the Pleistocene was followed by a short dry period which ended at about 9000–7000 bc. The treeless, sagebrush (*Artemisia*) steppe developed with a trend toward present conditions. It is quite possible that the dry climate and the steppe–desert environment were the direct cause of the withdrawal of the rich wild mammal fauna to the well forested and well watered mountains. Man tried to follow his wild prey, but with the decrease in the number of wild mammals hunting itself became less and less successful. At the same time, man found wild cereals in the mountains and began first to collect and then to plant them. This activity would certainly have required the settlement of at least one part of the population, although the hunters could have continued their seasonal wanderings. It seems more likely, however, that the scale was reduced to a simple change from a summer camp to a winter one. In consequence, however, the wild fauna around the settlement would have thinned out, and man—either consciously or spontaneously—had to look for new sources of animal protein. He found them in animal domestication.

It is, however, quite clear that demographic pressure must also have played an essential part in the changes in food production. Other causes such as the political pressure of more developed groups, the attraction of the products of already food producing communities, and so on, were only of secondary importance in this respect.

Consequently the main aim of domestication was—failing proper methods of meat conservation—to secure living meat reserves. Earlier authors saw the main aim of domestication as the production of enough sacrificial animals to the different gods. Some of these authors have even divided the early domestic animals into groups indicating whether they were the sacrificial animals of the sun god or the moon goddess. This theory certainly does not bear closer investigation, although it is true that some of the domestic species were used for sacrificial purposes at a rather early date. For example cattle horns were found in the shrine of Çatal Hüyük in the fifth millennium bc¹, and at about the same time dog hams were sacrificed by the mesolithic population of Lepenski vir in the Iron Gate gorge of the Danube².

Domestication did not start in one single centre. As man arrived at a particular stage of cultural development at the same time in many areas, he began to control and domesticate certain animal species. Thus each species was domesticated at about the same time in different places and what is designated the earliest site of domestication of a given species can be a matter of sheer chance, depending on where archaeological research has been most successful. On the other hand domestication did not cease after the acquisition of the first domestic animals. It played an important part in increasing domestic stock during the whole Neolithic, it existed in the course of later prehistoric periods, it occasionally appeared even in classical and medieval times and new species are still being domesticated in certain areas of the world.

For a long time it was almost axiomatic that domestication started with the advent of the Neolithic. In more recent times, however, there has been increasing evidence that its earliest beginnings go as far back as the Mesolithic or even late Palaeolithic. In these earlier periods, however, only isolated attempts occurred, with the taming and keeping of individuals of one single animal species that—in spite of all their successes in the domestication of the species in question—never resulted in real animal husbandry exploitation of several domestic species. All these attempts had a common characteristic: their subject was either the dog or (more rarely) the pig. Such animals, whose food requirements were very similar to those of

man, survived on the remnants of human food and needed no substantial vegetable fodder. It was not by chance that in the time of these attempts at domestication plant cultivation did not yet exist, and the idea of securing such fodder therefore could not arise.

Domestication can be traced back to the final period of the Palaeolithic also in the Near East. Turnbull and Reed found domestic dog remains in the Zarzian (final Pleistocene) layer (about 12000 bc) of the Palegawra Cave in North-east Iraq³. Among them is a lower jaw whose crowded premolars prove the domesticated character of the Palegawra dog, making it the earliest known domestic animal. Otherwise in all probability domesticated dogs were found in Natufian (10000–8000 bc) sites of Palestine⁴.

The next two species of the neolithic domestic fauna were acquired at the beginning of the Neolithic, in the “era of incipient cultivation and domestication” (to use Braidwood’s terminology⁵). This era signalled the end of the food collecting phase, when man populated a wide variety of environmental zones and “utilised a far greater variety of resources within any given environmental niche” than before. Flannery calls it a “broad spectrum” collecting pattern⁶. This era lasted from about 9000 bc to about 7000 bc and resulted in the domestication of both small ruminants, the sheep and goat, of our domestic fauna.

The small ruminants were ideal subjects for the first experiments of man in domestication. First of all the capture of their domesticable young was comparatively simple, for the killing of adult individuals (which tried to protect their young) was much less dangerous than the killing of an aurochs cow or a wild sow. Besides this, as small animals of non-predatory character, they could be more easily handled, even after they became adult in captivity. Another important point was that their development was much faster than that of the large species, and man could therefore see the results of his experiments sooner. Finally the unexacting food requirements of both sheep and goat could be of most importance in the conditions of primitive animal husbandry.

During this time man attempted the control of other small ruminants too; first of all the different gazelles. Enormous numbers of gazelles lived in the Near East in prehistoric times, and even today great numbers of them are still killed. They often live in large herds, and the possession and rational hunting of such a large herd could easily supply even substantial settlements with enough meat. Probably this was the case with Jericho in Palestine whose 2,000–3,000 early neolithic inhabitants could hardly get enough meat from the very small domestic fauna of the settlement. On the other hand, the large number of gazelle bones found in the site suggest well regulated gazelle hunting or possibly herding. But although herding can verge on the brink of domestication it does not necessarily develop into a real animal husbandry. (This was the case with the gazelles, in which morphological changes which could be ascribed to domestication have never been seen.)

The earliest supposed domestication of sheep was described by Perkins in Zawi Chemi Shanidar, North-east Iraq from 10,870±300 yr BP (ref. 7). The sheep of Shanidar do not show any morphological changes, and their domesticated nature has been inferred from the high ratio (about 50%) of the animals killed while still immature, the sudden increase in the proportion of sheep in the fauna and finally the fact that the region of Shanidar is not considered the natural habitat of wild sheep.

It is appropriate to go over the evidence for domestication at this point because the same problems arise in every case of early domestication. The best evidence for domestication is the appearance of morphological changes (such as a decrease in size, changes in the body propor-

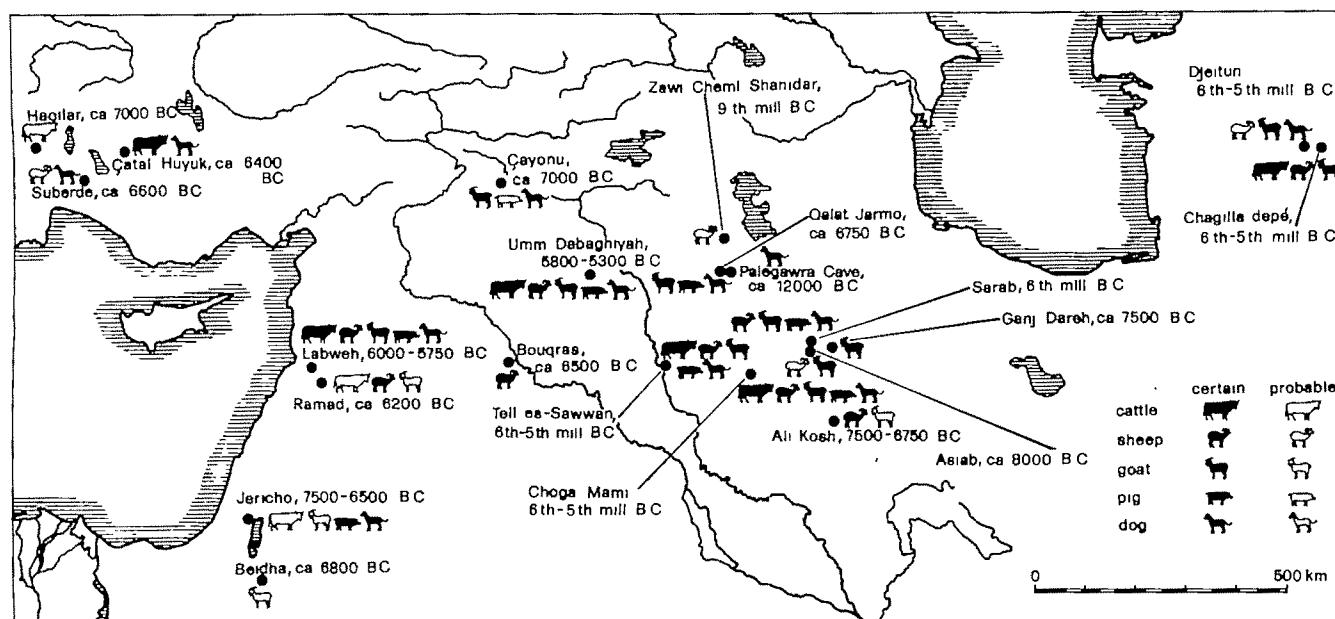


Fig. 1

tions and the form and fine structure of the bones, shortening of the skull, crowded or decreased teeth, hornlessness, changes in the horn form, and so on) in the animals. However, well defined morphological changes do not occur before about 30 generations, according to modern experiments on domestication. The length of a generation is 2–3 yr in small species (dog, sheep, goat, pig) and 5–6 yr in large species (cattle, horse, and so on). Since the earliest domestication of the first domestic species goes back as far as 8,000–14,000 yr, the delay of 60–90 or 150–180 yr in the occurrence of the morphological changes is hardly significant.

But there is indirect evidence of domestication which precedes the appearance of morphological change⁸. This evidence bears on the Near East, because the morphological differences between the wild and domestic forms of the ovicaprids, the most common species there, were not very well known even five years ago. In the late forties and early fifties the high ratio of immature animals alone was accepted as evidence of domestication in the Near East, but it is realised now that it can also be the result of certain hunting methods, and it is accepted as evidence only if it is corroborated by other data.

Further evidence of domestication is the high ratio of adult individuals and particularly of adult males among the remains of the wild form of a domestic species. The explanation is that in specialised hunting connected with domestication man first killed mature individuals, and tried to capture the young ones. At the same time the high ratio of killed males was due to the husbandry of the wild herd where man discovered early that fewer males than females are needed to keep the herd at a given level, and he therefore preferred to kill males if he needed meat.

Animal husbandry increases the occurrence of embryos and newborn animals and also tracks and excrement in settlements. Finally buildings and tools, equipment connected with animal keeping, and representations of domestication scenes of domestic animals, can also be very useful evidence.

Another possible occurrence of early domesticated sheep is in Asiab, West Iran, about 8000 bc (ref. 9). Morphological changes cannot be seen here either, but the very high ratio (80%) of mature animals and the exclusive occurrence of males in the wild sample suggest controlled hunting which might be connected with domestication. On the other hand, the excavation of Ali Kosh, again in

West Iran (7500–6750 bc) yielded remains of undoubtedly domesticated sheep, as well as the skull fragment of a hornless ewe¹⁰. (In prehistoric times there were no hornless individuals among wild sheep; such individuals appear only recently in small, isolated populations.) In the seventh millennium bc domestic sheep appear also in the northern Euphrates area (Bouqras, Ramad) and probably in Anatolia (Suberde) too¹¹.

The earliest domestic goats were found in Asiab, about 8000 bc¹². The occurrence of those domestic goats can be shown by anatomical changes (twisted horns) and the high ratios of mature (82%) and male (95%) individuals among the wild goats killed by the inhabitants. Just a little more recent is the appearance of the domestic goat at Ganj Dareh (7500 bc), in the immediate vicinity of Asiab¹³. The animal bone sample of this site has not yet been published in detail, but tracks found on the mud bricks of the site point to appearance of domestic goats in the settlement, since wild goats would surely not have come so close to human settlement. As for Ali Kosh, the occurrence of domestic goats in its earliest phase (7500–6750 bc) is uncertain, though probable¹⁴. The domestic goats of Jericho probably also originate from the eighth millennium bc (ref. 13). On the other hand, they can almost certainly be found all over the Near East.

The domestication of cattle and pig, the two latest species in neolithic animal husbandry, occurred during the seventh millennium bc¹⁵. In this era, villages produced most of their food, though hunting and gathering were still important in certain areas. This era began at the end of Prepottery Neolithic and grew into early Pottery Neolithic.

The earliest positively domesticated pigs in the Near East were found in Qalat Jarmo, about 6750 bc (ref. 15). They were fully developed domestic animals with well defined morphological changes. Since there are no traces of a local domestication in the site, one must suppose that they got there from another site where pig domestication took place earlier. The occurrence of domestic pigs is only probable in Çayönü, Anatolia (7000 bc)¹⁶ but it is certain in the early levels of Jericho¹⁷.

The earliest domestic cattle of the Near East are from Catal Huyuk, Anatolia (6400 bc)¹⁸. Some of the cattle bones of the aceramic level of Hacilar, Anatolia (7000 bc) may also be from domesticated animals (they seem to be smaller than the aurochs bones) but it is still uncertain because of the small sample size¹⁹. The occurrence of

domestic cattle in the pre-ceramic phase of Jericho is also not entirely certain¹⁷.

The above descriptions clearly demonstrate that man had completed the domestication of all five species characteristic of neolithic animal husbandry in the Near East by the middle of the seventh millennium BC. On the other hand, it is interesting that the five species did not appear together in any site in the area before the end of the seventh millennium BC, although many different species combinations occurred on many sites. What is particularly curious is that the same five species could be found in several pre-ceramic sites of the Greek islands and mainland Greece around the middle of the seventh millennium BC¹⁸⁻²⁰. This occurred in spite of the fact that out of the five species two (sheep and goat) could not be domesticated on the European mainland because of the absence of wild forms, while two others (dog and pig) were domesticated in the Near East earlier than in South-east Europe. With only one (cattle) does European domestication seem to precede the Near Eastern, although with such a slight time difference (the radiocarbon date of Argissa Magula, Thessaly is 100 years older than that of Catal Hüyük, Anatolia) that one cannot be sure. Nevertheless, the final word has not yet been spoken on this question because animal bone samples from the pre-ceramic West Anatolia have not yet been published. This region shows the closest genetic connections with the earliest Neolithic of South-east Europe.

As mentioned above, animal husbandry embracing all five domestic species developed in the Near East at the end of the seventh millennium and became widespread during the sixth millennium BC. From this period the faunas of several sites are known, for example Labweh from Lebanon, Umm Dabaghiya, Tell es-Sawwan and Choga Mami from Iraq, Sarab²¹ and Tepé Sabz¹⁰ from Iran, Djeitun and Chagilla depe²² from Turkmenistan. Their common characteristic is that the caprovines occur as an overwhelming majority in them; the ratios of the other domestic species are rather variable. The ratios of sheep and goat were determined by the geographical environment: in mountainous regions the goat was more common, on plains and among hills the sheep were in the majority. In the settlements of this period also the importance of animal husbandry relative to hunting was quite variable. In general, animal husbandry was more important, but in certain areas hunting remained of decisive importance. In such cases it took a specialised form, such as in Sarab where it was connected with goat domestication, or as in Umm Dabaghiya without local domestication. This latter site is particularly important because, in spite of the fact that its inhabitants kept all five neolithic domestic species, the importance of animal husbandry was minimal, the remains of domestic animals comprising only 10-12% of the animal bone sample. The hunting was specialised for the half-ass (onager) and its bones represent nearly 70% of the sample. This overspecialised hunting did not, however, lead to onager domestication.

The neolithic domestic fauna did not change much even in the further course of the period, and only the sheep gained more ground against the goat. On the other hand, the importance of animal husbandry grew at the expense of hunting which generally declined after the fifth millennium BC. In the third to fourth millennia, the horse and the ass joined a domestic fauna that was gradually completed by new species later, none of which, however, endangered the leading position of the caprovines.

Concerning the early domestic animals of the Near East one may reasonably ask what use they were to neolithic man. As already hinted their original use was almost exclusively for meat. The fact that the first domesticated animals—with the exception of the dog—were derived from species which were originally hunted for their meat has obvious implications. Furthermore, early neolithic man killed most of his domestic animals at an immature age, for example,

caprovines before the first or second winter. The reason was not only that the meat of young animals was more palatable but also that man could provide fodder only for a small, precious breeding stock in the winter fodder shortage. In the advent of domestication and animal husbandry man ate the flesh of every domestic species including the dog whose skulls with opened brain-case (brain was considered a delicacy even in those times) can often be found in the garbage pits of settlements up to the Bronze Age²³. Man did not cease to eat dog meat until the end of the Bronze Age—probably the earliest food prohibition in the world.

The secondary use of hide or fur, tendons, intestines, bones and so on, for tools and clothes was probably established quite early. Only the dog, however, had secondary uses as a live animal, in the roles of hunting companion, watch-dog and herd-dog. In the European Mesolithic it was also used as a sacrificial animal²⁴, although this is not yet proven for the Near East. On the other hand, cattle sacrifices in the Near East have very ancient origins. In the shrine of Catal Hüyük both aurochs and cattle horn-cores were found embedded into clay animal heads²⁵, while the bull cult goes back as far as the sixth millennium BC²⁶.

Among real secondary uses of live animals probably milking was the earliest. Animals at a primitive stage of domestication had obviously only enough milk for feeding their young, although they could be milked if their young died or were slaughtered. As prehistoric bone samples witness, death of the young animals was not rare in early animal husbandry, and the young were probably slaughtered so that the suckler animals could be milked (this might also account for the high ratio of animals killed as juveniles in prehistoric settlements). Later, enough milk was produced to give a surplus for human consumption. It is obviously difficult to demonstrate milking from prehistoric material. The earliest representation of milking and milk processing is known from a frieze of the Nin-Hursag temple in Ur (after 2400 BC)²⁷. Interestingly enough the cows are being milked in this scene from the rear, as is usual with goats. From this one may infer that the milking of cows may have emerged after the milking of goats.

Another early secondary use was that of wool of sheep. The body of the wild sheep is covered by coarse kemp among which wool fibres appear only in winter time. These wool fibres are, however, very short and straight. Long, curly wool fibres which do not undergo yearly changes appeared apparently as a result of sudden mutation after domestication. This mutation probably occurred rather early, to judge from a clay sheep statuette found in Sarab (sixth millennium BC)²⁸ on which the artist has depicted very skilfully the curliness of the wool. Such representations became very common from the fourth millennium BC on, and clay tablets from the turn of the third and second millennium BC often gave sheep shearing lists from estates of Old-Babylonian temples²⁹.

It is still not known how far back the use of draft power of cattle goes. Since there are osteological data in this respect from the Neolithic of South-east Europe, however, one can assume the same too on the Near East. Wheeled vehicles appeared there in the fourth to third millennium BC and they were drawn by cattle. In view of the highly developed state of agriculture in Mesopotamia, cattle-drawn ploughs or some kind of unwheeled vehicle (travois, sledge) could easily have occurred.

The development of early animal husbandry came to an end in the third millennium BC, and at the turn of the third and second millennia, a qualitative change took place. A switchover started from primitive animal keeping to selective conscious animal breeding. This happened on the estates of temples first and first concerned only wool-bearing sheep but later extended slowly to other species as well. As a result of this the size and production of domestic animals grew, their body proportions changed, and new breeds developed which reached remote regions in a short

time and exerted an influence on the domestic animals and animal husbandry as a whole.

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articles

Retrogression rate of the Victoria Falls and the Batoka Gorge

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From an analysis of the archaeological dates for deposits in the Victoria Falls area, we suggest that the rate of erosion of the Victoria Falls was somewhat slower than previously assumed.

ATTENTION was first drawn in the pages of *Nature* in 1905 (ref. 1) to the evidence for the late date and relatively fast rate at which the line of the Victoria Falls, straddling the Zambezi river between Zambia and Rhodesia, reached its present position. In spite of initial challenges, the view was confirmed by subsequent geological and archaeological studies^{2,3} that the gravels now isolated from 110 m to >250 m above the river were laid down by the Zambezi before and during the gradual retrogression of the falls.

The successive positions of the line of the falls are considered to have followed zones of close jointing which formed belts of weakness in the basalt bedrock. These formed steep sided gorges, visible for ~111 km downstream from the Victoria Falls. The archaeological contents of these river-laid deposits above the gorges, or of deposits and events considered to be contemporary, have been used to determine the date by which the falls line had reached different positions⁴, and thus to calculate the speed of retrogression. There are clearly limits to the reliance that can be given to the geological dating of deposits by their archaeological contents, even where these do not seem to be redeposited. In the absence of much needed new geomorphological studies, however, the archaeology remains our best source of dating evidence. The speeds of retrogression previously calculated^{4,5} gave figures which are equivalent to rates of regression between 3,380 and 5,930 yr km⁻¹.

Such a fast rate of retrogression contrasts with the subjective impression of stability of the falls gained by residents

and visitors. While rock fall is very infrequently observed at the falls line itself, in spite of a mean annual water flow of $3.6 \times 10^9 \text{ m}^3 \text{ yr}^{-1}$, it seems no greater at the line of the western end of the falls, which has been identified⁶ as the beginning of the next line of falls.

The rate of erosion of the gorges was not constant, because of the variable section of the channel in the gorges, and the climatic variation of rainfall in the basin of the upper Zambezi in eastern Angola, western Zambia and northern Botswana. A mean post-Pleistocene rate of 0.29 m yr⁻¹, which could be derived from suggestions⁷ for the date of the erosion of the upper gorges, would, however, seem to require more visible modern erosion.

An empirical test may be made from a generally very accurate topographical illustration by Thomas Baines who visited the falls in 1862. One of his paintings⁷ shows the beginning of the 'new gorge' from the position of the western end of the first gorge. Comparison with the present condition suggests erosion here could not have been more than a few metres in over a century. The view shown represents what today⁸ is a stretch of ~75 m.

Recent reassessments of the dating of Stone Age industries of Southern Africa suggest that the dating of the retrogression of the Victoria Falls based on such industries can now also be reconsidered.

The upper gorges

The date of the erosion of the Fourth Gorge is considered⁹ to follow the Middle Stone Age occupation of the area. The Kalahari Sand II, actually redeposited Kalahari Sands laid down in a dry period, overlies river-bed gravel to the south of the Fourth Gorge, but not further upstream. This sand, contemporary with what was formerly called the 'Rhodesian Magosian' industry, was laid down before the erosion of the upper three gorges.

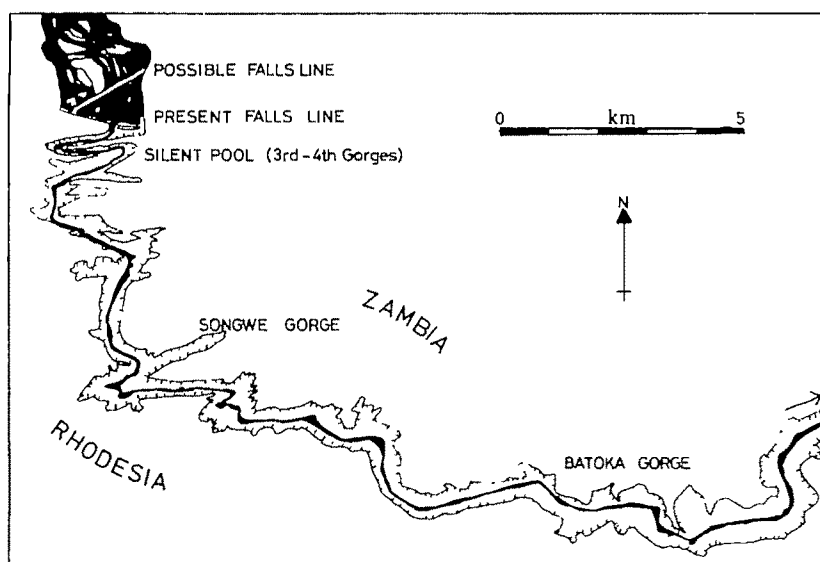


Fig. 1. Map of the falls area.

The distance from the Silent Pool, where the fourth gorge meets the third gorge, to the present day falls is 2.96 km (ref. 8) while the distance from the exit of the second gorge into the falls line to the beginning of the projected new gorge at the western end of the falls is a further 1.17 km, a total of 4.13 km linear retrogression. Estimates of the rate of retrogression have used the north-south erosion following the river, but not the easier lines of erosion which transect this line, and we can therefore use the figure of 2.96 km.

The dates of Magosian and related industries have been reassessed recently. They can no longer be seen as late or terminal Pleistocene cultures making the transition of Middle to Later Stone Age, but rather a long term tradition partly contemporary with other Middle Stone Age industrial traditions⁹. Sites attributed to the Magosian complex and related industries in South Africa have been ¹⁴C dated¹⁰, from $>44,500$ to $>50,250$ yr b.p. at Rose Cottage Cave, and $45,900 \pm 2,100$ yr b.p. at Montagu Cave, but with other dates at the end of the Pleistocene⁸. In Rhodesia dates for Magosian-related material may be later; Pomongwe has a date of $15,800 \pm 200$ yr b.p. Finds in Zambia which have been grouped with those from the Kalahari Sands II¹¹ include assemblages of 'Proto' Later Stone Age¹² from Leopards Hill dated between $23,800 \pm 360$ yr b.p. and $21,550 \pm 950$ yr b.p. Apparently contemporary is the 'Pre-Later Stone Age' industry at Kalembo in eastern Zambia¹³ dated to 24,600 yr b.p. and 24,420 yr b.p., it is succeeded by a Later Stone Age industry dated to at least $15,330 \pm 1,100$ yr b.p., while a similar industry at Leopards Hill is dated to $16,715 \pm 95$ yr b.p.

At Kalambo Falls in northern Zambia a Magosian-related industry named the Polungu¹⁴ has been dated by J. D. Clark at more than $9,550 \pm 210$ yr b.p., possibly as old as 20,000 yr b.p., or even, by comparison with finds at Zombepata Cave¹⁵ some 20,000 yr earlier than that. At Kalambo Falls, however, an earlier industry of Lupembian type has been dated at $31,660 \pm 600$ yr b.p., but this date seems younger than should be expected on other grounds; a date over 70,000 yr b.p. for most of the Lupembian may be more probable (K. W. Butzer, personal communication).

Present evidence would suggest the demise of Magosian and related industries in most of Zambia and Rhodesia was considerably earlier than the end Pleistocene date of 12,000 b.p. previously assumed. The most likely time is between 21,000 and 17,000 b.p. with an origin for the complex in the area between 25,000 b.p. and 30,000 b.p. There is as

yet little to support for Zambia or for Rhodesia¹⁰ a date similar to the estimate for South Africa^{10,17} that the Later Stone Age began at $\sim 35,000$ –20,000 yr b.p. The question of such a date will probably become less conflicting when more detailed study has been carried out on the nature of the industries in the 40,000–12,000 yr b.p. range.

The Kalahari Sands II which predate the erosion of the last 2.96 km of gorge, are therefore dateable to a dry phase within the period defined for the Magosian, at the outside 30,000–17,000 yr b.p., and more probably 30,000–20,000 yr b.p.

Correlated rock falls in Rhodesian caves with archaeological deposits¹⁸ suggest there was a wet period following the end of the 'Stillbay' phase of the Middle Stone Age, and dated at between 25,000 and 21,000 yr b.p. It was followed by a dry phase in which the local variant of the Magosian flourished, a period one can correlate with the Kalahari Sands II of the Victoria Falls, which would thus be dated at later than 25,000 yr b.p. Palaeoclimatic studies at Kalambo Falls¹⁸ suggested close correlation of the temperature curve with that of Europe during late Pleistocene and post-Pleistocene times but the rainfall pattern need not be correlated with temperature change.

Accepting the suggested date limits for the Zambian industries related to the Magosian, and the Rhodesian rock fall, a date for the deposition of the Kalahari Sands II would be between 25,000 and 17,000 yr b.p.

If we place the termination of the Zambian Middle Stone Age at the same time as that now suggested for South Africa, this would allow 20,000 b.p. as a possible date for the start of the erosion of the third gorge, or a mean rate of $6,760 \text{ yr km}^{-1}$ (0.15 m yr^{-1}).

The Zambezi to Songwe Gorge

The dating of the gorges below this point relates to the Younger Gravels and associated earlier deposits attributed to the Upper Pleistocene. The younger gravels themselves contain derived Early Stone Age material and less abraded material from the beginning of the Middle Stone Age, formerly called 'Proto-Stillbay'. The overlying sands and calcretes have a 'Stillbay' industry. Such younger gravels are found at least as far as the top of the gorge above the confluence of the Songwe river with the Zambezi, some 6.75 km below the turn of the fourth gorge into the third, measured along the line of the modern river.

The Younger Gravels were claimed to have been laid

down in a period of high rainfall originally identified with the 'Third Pluvial'.

The river above Songwe Gorge must have eroded back since the period of high rainfall and earlier Middle Stone Age^{4,5}; this period had previously been estimated as within the past 50,000–40,000 yr.

The beginning of the Middle Stone Age in Southern Africa has been suggested at ~100,000 yr b.p. (ref. 10) and perhaps over 130,000 yr b.p. as indicated by recent work (K. W. Butzer, personal communication). At Nelson Bay Cave on the Southern Cape coast of South Africa, the Middle Stone Age extended back into the Eem Interglacial probably before 75,000 b.p. (refs 19, 20) and very possibly before 125,000 yr b.p. (K. W. Butzer, personal communication).

The earliest Middle Stone Age radiocarbon date for Zambia is $31,660 \pm 600$ yr b.p. at Kalambo Falls, although the preceding Sangoan was originally dated only six centuries before¹⁴, with an early date of 46,100 yr b.p. Other forms of dating contrast with this picture. This is especially true of the finds of Broken Hill Man at Kabwe in central Zambia. Amino acid racemisation dates for human and associated animal bones at Broken Hill suggest a date of ~110,000 yr BC for Broken Hill Man^{11,22}. Klein²³ argues that the faunal remains probably associated with Broken Hill Man must fall within the Eem Interglacial, or in the Middle Pleistocene (which would imply an earlier date).

The archaeological associations with these dates are unfortunately not completely clear. At the time of Clark's classic study of the Stone Age cultures of the Zambezi valley, however, he attributed³ the finds with Broken Hill Man to 'Proto-Stillbay', and thus a parallel is suggested—whatever terminology be accepted today—between the Broken Hill finds and those of the Younger Gravels. Subsequent reassessment, however, suggests²² the associations may be Sangoan.

The 'Proto-Stillbay' industry was seen as derivative from Sangoan in the Victoria Falls area. It seems, however, that Sangoan industries in southern Africa as a whole overlapped with the earliest Middle Stone Age⁹. The Sangoan at Kalambo Falls was dated by radiocarbon between 46,100 and >32,600 yr b.p., and the preceding Acheulian between >35,000 and $61,700 \pm 1,300$ yr b.p. but these dates now seem much too late. A new minimum date from amino-acid racemisation of >110,000 b.p. (ref. 24) for the Acheulian of the site suggests all the earlier dates for Zambia need to be held in some uncertainty, and probably accepted as *termini ante quos*. The early Middle Stone Age industry of the Younger Gravels at Victoria Falls, which derives from a Sangoan industry, cannot be dated later than 40,000 yr b.p. while in the light of the new dating evidence, a date of <130,000 yr b.p. seems acceptable.

Correlation with the Broken Hill bones and artefacts would suggest a date at ~110,000 yr b.p. for the artefacts of the Younger Gravels. The geological chronology from the southern African coast¹⁹ suggests that the main wet period within the range of the early Middle Stone Age in that area was in the Eem Interglacial before 75,000 yr b.p. Correlations of coastal and inland rainfall patterns are not necessarily reliable, and it may therefore be more useful to rely on the cultural data. Since the erosion above the Songwe Gorge is subsequent to the earlier Middle Stone Age settlement, the falls may have taken up to 90,000 yr to cut back the 6.75 km from the Songwe river to the third gorge, a maximum rate of $13,330 \text{ yr km}^{-1}$ or an average of $11,280 \text{ yr km}^{-1}$ for the past 110,000 yr.

If the retrogression of the stretch from Songwe Gorge were taken to follow the end of the Interglacial, at 75,000 yr b.p., the rate from Songwe to the fourth gorge would be $8,150 \text{ yr km}^{-1}$; an average for the whole distance of $7,725 \text{ yr km}^{-1}$. These alternatives correspond to an average rate of 0.09 or 0.13 m yr⁻¹.

The lower gorges

The area of the gorges below Songwe Gorge has poor access and has not been studied in any detail. The gorges extend ~101 km measured along the river, from the Songwe river to their limit above the confluence with the Matetsi, and the dating of the erosion of this stretch of river is hard to determine. Clark³ originally considered that the gorge, perhaps to 6 km above Ohimamba Rapids (a distance that can be calculated at 31 km along the river) was eroded in the Upper Pleistocene, while the lower gorges (70 km) were eroded in the Middle Pleistocene; but a Lower Pleistocene date for the lowest part of the gorges was subsequently suggested⁴. There seems little direct geological evidence for this, and no archaeological data is yet available for the area, although further fieldwork would be useful.

The Middle Pleistocene is now dated²⁸ from 700,000 to 130,000 yr b.p. If we adopt the alternative rates calculated for the retrogression from Songwe Gorge to the modern falls line in the last 75,000 or 110,000 yr, the erosion of the remaining 31 km of the stretch above Chimamba Rapids would have taken 240,000–350,000 years, since 315,000–460,000 yr b.p., well within the Middle Pleistocene.

The presence of Older Gravels of possibly early Middle Pleistocene date near the Matetsi confluence at the lower end of the whole gorge suggests a Lower Pleistocene date for the beginning of the falls retrogression. It seems likely that the erosion of the gorge above the Chimamba Rapids was much more recent than the lower stretch of 70 km (ref. 3), and the geomorphology of the slope and the available evidence on river-laid deposits for this stretch suggests a possible chronological break between the erosion of the lower 70 km and the upper stretch. It is tempting to suggest that this break may have been in the early Middle Pleistocene. The lower stretch from the Matetsi confluence above the Chimamba Rapids, which has geomorphological unity, could all have been eroded within the Lower Pleistocene, over a period (calculated from the rates for the upper stretch) between 540,000 and 790,000 yr since at least 1.25×10^6 yr b.p., an average of 0.9 m yr^{-1} .

In the absence of well dated contemporary sequences in the region, archaeological dating of the geological events can only be tentative and more detailed geomorphological studies, especially on the lower gorges of the Zambezi, would do much to clarify the situation.

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Transformation by murine and feline sarcoma viruses specifically blocks binding of epidermal growth factor to cells

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Normal cells in culture have membrane receptors for epidermal growth factor (EGF); EGF stimulates cells to divide by binding to these receptors. Cells transformed by murine and feline sarcoma viruses rapidly lose the ability to bind EGF, whereas cells transformed by the DNA tumour viruses, polyoma and SV40, or infected with non-transforming RNA tumour viruses have normal levels of functional EGF receptors. The results suggest that a product of the sarcoma virus genome specifically changes cell EGF receptors; the sarcoma gene product may, then, be functionally related to EGF.

RNA-containing mammalian and avian sarcoma viruses (type C viruses) can produce tumours in a wide variety of susceptible animal hosts and can transform cells from a number of different species in tissue culture^{1,2}. Genetic analyses using temperature-sensitive mutants³ have distinguished specific viral genes (sarcoma genes or oncogenes^{4,5}) essential for transformation. The genomes of the sarcoma viruses contain specific RNA sequences⁶⁻⁸ which are not found in transformation-defective type C viruses. The sarcoma-specific information could code for a protein with a maximum molecular weight of 30,000–40,000. The sarcoma gene product, however, has not been identified; it is presumably synthesised in small quantities and is biologically highly potent. Sequences related to the sarcoma-specific genes have, however, been found as normal constituents of the cellular DNA of both birds⁹ and mammals^{10,11}. From these considerations, we would suggest that the product of the genes should be related to or identical with a normal cellular gene product and that such a protein is (1) a regulator of cell proliferation, (2) of low molecular weight, (3) evolutionarily conserved, (4) produced in only small quantities by sarcoma virus-transformed cells, and (5) synthesised by some normal cells which have not been infected by sarcoma viruses.

One protein that satisfies the criteria for a possible oncogene product is epidermal growth factor (EGF). Mouse epidermal growth factor has 53 amino acids and a molecular weight of 6,045 (ref. 12). It will stimulate the proliferation of various epidermal and mesodermal cells in both whole animals and tissue culture. Injection into neonatal mice accelerates the time of eyelid opening and tooth bud eruption^{13,14}. In organ culture EGF stimulates the growth of epithelial, corneal and mammary gland cells¹⁴⁻¹⁶ and in tissue culture it stimulates cell division of mouse cell lines such as 3T3 and BALB/3T3^{17,18}, mouse and human diploid fibroblasts^{13,19} and human glial cells²⁰. As little as 0.1 ng ml⁻¹ of EGF will stimulate DNA synthesis in human fibroblasts²¹. A closely related protein (human EGF) has also been puri-

fied from human urine and shown to have all the biological properties of mouse EGF^{21,22}.

The primary event in growth stimulation induced by EGF (as with other polypeptide hormones²³) is the binding of EGF to specific cell membrane receptors^{24,25}. The factors from both species utilise the same set of membrane receptors in a wide variety of vertebrate cells for their growth stimulatory effects²¹. The genes for both EGF production and its membrane receptors, therefore, seem to have been well conserved in evolution, although the precise normal physiological function of this growth regulatory system is not fully understood.

We now report that in several cell culture systems transformation by mouse and feline sarcoma viruses produces a rapid and profound loss of detectable membrane receptors for EGF. This is not seen in cells transformed by the DNA viruses, SV40 and polyoma, or in cells producing non-transforming leukaemia viruses.

Mouse EGF binding and transformation

Table 1 shows the binding of ¹²⁵I-labelled EGF to various normal and transformed cells in tissue culture. Three different clonal systems of mouse cells were available for study—random bred Swiss 3T3 (ref. 26), BALB/c 3T3 (ref. 27), and NIH Swiss 3T3 (ref. 28). Clonal lines of parental cells were compared with transformed clones produced either by DNA-containing viruses or by RNA-containing viruses. Cells were assayed in their log phase of growth when they were actively dividing, 24–48 h after seeding subconfluent cultures. In each mouse cell system, the Moloney sarcoma virus-transformed cells²⁹ as well as the Kirsten sarcoma virus-transformed cells³⁰ were much less able to bind labelled EGF. One Rous sarcoma virus-transformed BALB/3T3 clone (B77-A31 clone 1) showed only a slightly reduced level of binding compared with normal cells and was quite different in this respect from the MSV-transformed cells.

The 3T3 cell line has been shown to be susceptible to transformation by two DNA tumour viruses, polyoma and SV40 (ref. 31), and, further, doubly transformed clones of 3T3 could be obtained that had integrated both SV40 and polyoma genomes^{32,33}. These cells have all the *in vitro* properties associated with viral transformation and produce tumours in susceptible animal hosts. Whereas MSV-transformed 3T3 clones bind very little labelled EGF the DNA virus-transformed cells show levels of binding not significantly different from the parental cells (Table 1).

When cell lines derived from rat (NRK cells³⁴), mink (Mv1Lu cells³⁵), and cat (FFc60WF³⁶) were studied, cells transformed by the murine sarcoma viruses again showed greatly reduced binding of labelled EGF (Table 1). The normal mink kidney cells, an epithelial line, bound approximately 3–5 times more labelled EGF than did 3T3,

Table 1 Binding of mouse ^{125}I -EGF to normal and virus-transformed cells

Cell line	Transformed by	Designation	Binding ^{125}I (c.p.m. per 10^6 cells)
BALB/3T3	None SV40	A31-CL7	6,700
		SV-A31-CL1	6,900
		SV-A31-CL3	8,700
		SV-A31-CL6	5,900
		M-A31-CL71	310
	MSV-Moloney MSV-Kirsten RSV-B77	K-A31-CL234	140
		B77-A31-CL1	4,300
		A31 (producing R-MuLV)	7,400
		3T3-CL4	4,700
		SV-3T3-CL6	4,300
Swiss 3T3	None	PY-3T3-41	5,200
	SV40	SV-PY-3T3-11	4,600
	Polyoma	M-3T3-CL4	230
	SV40 and polyoma	NIH/3T3	4,800
	MSV-Moloney	NIH/3T3 (producing R-MuLV)	8,300
NIH Swiss	None	SV-NIH/3T3-CL1	6,500
	None	SV-NIH/3T3-CL6	7,200
	SV40	M-NIH/3T3-CL5	200
	MSV-Moloney	K-NIH/3T3-CL65	100
	MSV-Kirsten	NRK-CL2	7,600
	None	M-NRK-CL5-3	230
	MSV-Moloney	K-NRK-CL1	60
	MSV-Kirsten	FSV-NRK-CL3	320
	FSV-Gardner	V-NRK-CL2 (spontaneous rat type C virus producer)	9,800
	None	Mv1Lu (CCL 64)	25,800
Mink lung	None	M-MSV-64-CL7	3,200
	MSV-Moloney	K-MSV-64-CLJ1	2,300
	MSV-Kirsten	FSV-64-CL2	2,800
	FSV-Gardner	FFc60WF	2,600
Cat embryo	None	MSV-FFc60	280
	MSV-Moloney	FSV-FFc60	230
	FSV-Gardner		

The binding assays were performed on subconfluent cell culture monolayers in 60-mm Petri dishes (Falcon 3002). Approximately 1×10^6 cells per dish were seeded in Dulbecco's modified Eagle's medium (DMEM) containing 10% calf serum 24 h before binding ^{125}I -EGF. The cells were washed twice with 2-ml aliquots of binding buffer which consists of DMEM containing 1 mg ml $^{-1}$ bovine serum albumin (BSA) and 50 mM *N,N*-bis-(2-hydroxyethyl)-2-aminoethanesulphonic acid (BES), adjusted to pH 6.8. Binding was performed using 3 ng of ^{125}I -labelled mouse EGF (containing approximately 110,000 c.p.m.) in 1.5 ml of binding buffer. After incubation for 40 min at 37 °C, unbound ^{125}I -EGF was removed and the cells washed four times with 2-ml aliquots of cold binding buffer. The cells were solubilised in 0.75 ml of lysing buffer, composed of 0.01 M Tris-HCl, pH 7.4, containing 0.5% sodium dodecyl sulphate and 1 mM EDTA, and the contents transferred to a counting vial. The dishes were washed two more times with 0.5-ml portions of lysing buffer, the contents transferred to the counting vial, and the radioactivity determined using a Beckman 300 series gamma counter. Nonspecific binding was estimated by determining the amount of cell-bound radioactivity in the presence of 10 μg of unlabelled mouse EGF. All data given have been corrected for nonspecific binding. In a typical experiment where 10^6 c.p.m. of ^{125}I -EGF was added, the values for nonspecific binding range from 50–150 c.p.m. above machine background. To test for the possibility that the sarcoma virus-transformed cells rapidly degrade the labelled EGF by proteolytic action, normal and transformed cells were cocultivated in varying ratios and assayed for EGF binding. The binding to normal cells was independent of the number of K-NRK cells present even at a 50-fold excess of transformed cells (2×10^6 normal cells to 1×10^6 transformed cells). When ^{125}I -labelled EGF was first incubated with transformed cells for 60 min and then transferred to normal cells it bound with the same efficiency as freshly-added EGF; no significant degradation of EGF by the transformed cells could be detected.

NRK or cat cells. Although the transformed mink cells bound detectable quantities of labelled EGF, considerably less polypeptide was bound by transformed clones compared with the normal cells (85–95% reduction). Feline sarcoma virus, FeSV (Gardner-Arnstein strain²⁷), transforms the rat, mink and cat cells; non-producer transformed clones have been obtained in all three systems (unpublished).

Cells transformed by FeSV also showed greatly reduced EGF binding, similar to the levels of binding seen with cells transformed by the murine sarcoma viruses. A clone of NRK transformed by avian sarcoma virus (Schmidt-Ruppin strain) showed more nearly normal levels of EGF binding. Reduction in EGF binding to cells derived from several mammalian species is evident after infection by

Table 2 Binding of human ^{125}I -EGF to normal and virus-transformed cells

Cell line	Transformed by	Designation	Binding ^{125}I (c.p.m. per 10^6 cells)
BALB/3T3	None	A31	3,100
	MSV-Moloney	M-A31-CL71	300
	MSV-Kirsten	K-A31-CL234	110
	SV40	SV-A31-CL1	6,800
	SV40	SV-A31-CL6	4,300
NRK	None	NRK-CL2	7,900
	MSV-Moloney	M-NRK-CL537	70
	MSV-Kirsten	K-NRK-CL1	10
	FSV-Gardner	FSV-NRK-CL3	190
Mink lung	None	Mv1Lu (CCL 64)	24,500
	MSV-Kirsten	K-MSV-64-CLJ1	1,300
	FSV-Gardner	FSV-64-CL2	900

The binding assays were similar to those described in Table 1 except that ^{125}I -labelled human EGF (1.2 ng, 63,100 c.p.m.) was used instead of ^{125}I -labelled mouse EGF. Ten micrograms of unlabelled mouse EGF were used as controls for these experiments. As in Table 1 the nonspecific binding was 50–150 c.p.m. above the counter background.

MSV and FeSV, but not by avian sarcoma viruses or transforming DNA viruses.

The replication of non-transforming type C leukaemia viruses does not affect the ability of cells to bind EGF. Both BALB/3T3 (clone A31) and NIH Swiss 3T3 cells infected with MuLV (Rauscher strain) bind as much ^{125}I -labelled EGF as uninfected cells. Similarly, mink kidney cells infected with feline leukaemia virus²⁷ or with endogenous baboon virus^{28,29} show no reduction in EGF binding (data not shown). Clonal lines of mouse and rat cells such as subclones of A31-7 and NRK clone 2 that can spontaneously produce endogenous type C viruses also bind ^{125}I -EGF as effectively as their parental cell counterparts.

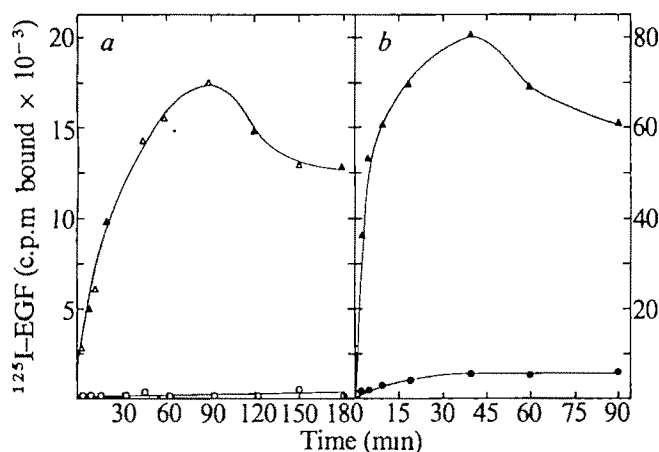


Fig. 1 Time course for the binding of ^{125}I -mouse EGF to normal and transformed cells. The binding buffer consists of Dulbecco's modified Eagle's medium (DMEM) containing 1 mg ml^{-1} of BSA and 50 mM N,N -bis-(2-hydroxyethyl)-2-aminoethanesulphonic acid (BES), pH 6.8. The bindings were carried out on cell monolayers in 60-mm plastic Falcon Petri dishes after washing the plates twice with 1.5 ml of binding buffer. The bindings were then started by the addition of 1.5 ml of binding buffer containing 15 ng of ^{125}I -mouse EGF ($5 \times 10^6\text{ c.p.m.}$) and incubated at 37°C for the stated periods. At the end of the incubations the binding buffer containing the unbound ^{125}I -EGF was removed and the cell monolayers were washed four times with cold binding buffer to remove the excess unbound ^{125}I -EGF. The amount of ^{125}I -EGF bound to the cellular monolayers was measured by lysing the cells and determining the radioactivity present in the lysate. *a*, Binding to normal rat kidney cells: the untransformed parent (Δ), NRK (6.0×10^5 cells per dish) and the Kirsten sarcoma-transformed cell (\circ), K-NRK CL1 (8.5×10^5 cells per dish). *b*, Mink cells: the normal parent (Δ), MvLu (4.5×10^5 cells per dish) and the Kirsten sarcoma-transformed cell (\bullet), K-MSV-64 CL1 (5.5×10^5 cells per dish).

Human EGF binding

An epidermal growth factor has been isolated from human urine which shows biological properties similar to those of mouse EGF^{21,22}. Table 2 shows that human EGF also readily detects the difference between MSV-transformed cells, normal cells and SV40-transformed cells. Like mouse EGF, more human EGF binds to the mink lung cells than to BALB/c 3T3 cells and NRK cells. Antisera to mouse EGF cross reacts only weakly with human EGF, and likewise antisera to human EGF cross reacts quite poorly with mouse EGF⁴⁰. Nevertheless, both mouse and human EGF can compete equally well with each other in the binding assays used here with mouse, rat and mink cells, suggesting that the biologically active portion of the molecule involved in binding to receptors has been more conserved during evolution than the major antigenic determinants recognised by antibodies prepared to the mouse and the human EGF.

Time course of EGF binding

Figure 1a shows the extent of binding of labelled mouse EGF to NRK cells, and to NRK cells transformed by murine sarcoma virus, as a function of the time of addition of labelled polypeptide. Maximal binding is achieved for NRK cells at 90 min, after which there is small decline in the EGF bound. This decline is not seen when the binding assays are performed at 23°C rather than 37°C . At no time during the 3-h incubation period at either temperature (in some experiments incubations have been run for as long as 5 h) is there any significant binding of EGF to the sarcoma virus-transformed rat cells. With mink kidney cells (Figure 1b), the time course is slightly different, with peak binding reached earlier, at 40 min, followed by a slight decline. With the transformed mink cells, however, there is a low level of EGF binding, again reaching saturation at 40 min. The transformed mink cells retain some receptors capable of binding EGF even after MSV transformation whereas, with rat and mouse cells, binding is almost entirely abrogated by MSV transformation.

To study the effect of EGF concentration on binding, an experiment was performed using increasing quantities of unlabelled EGF (up to 670 ng of EGF per 10^6 cells) together with the ^{125}I -labelled polypeptide (Fig. 2). No significant binding to the transformed NRK cells was seen at any concentration tested (data not shown). The final extent of binding to mink cells was maximal at approximately 850 pg bound per 10^6 cells for the normal mink cells and 8 to 9 times less for the transformed mink cells. Using this data to obtain a Scatchard plot, recognising that the system is not in complete equilibrium, we calculated that there are 8.4×10^4 receptors per normal mink cell and only 1.0×10^4 receptors per cell in the transformed mink clone. Detectable levels of binding to receptor sites on the transformed and normal cells can be completely inhibited by the addition of unlabelled EGF. Thus, with each of the

Fig. 2 Effect of EGF concentration on binding to normal and transformed mink kidney cells. Three nanograms of ^{125}I -EGF ($210,000\text{ c.p.m.}$) were used in each assay, and unlabelled EGF was added to bring the total EGF to the indicated concentrations. The specific binding was determined after a 40-min incubation at 37°C . Δ , Untransformed MvLu; \circ , Kirsten sarcoma virus-transformed progeny K-MSV-64 CL1.

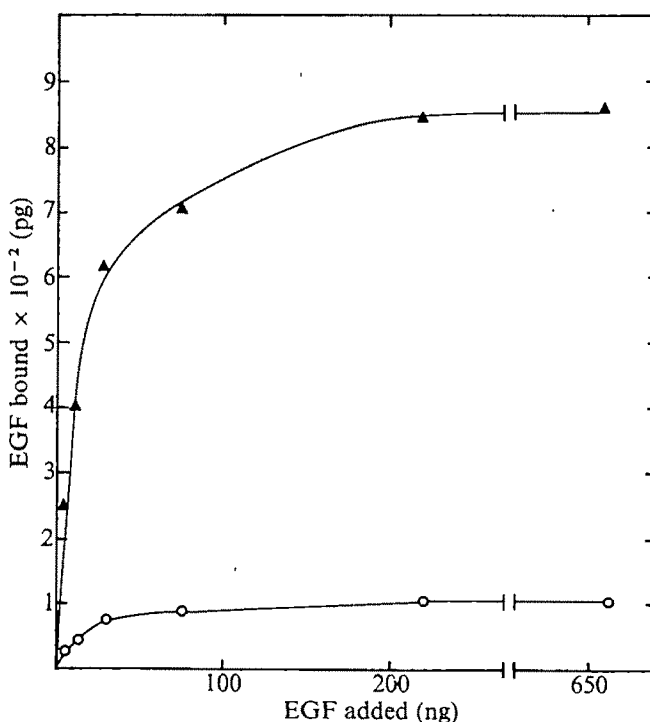


Table 3 Specificity of binding of ^{125}I -EGF to sarcoma virus-transformed cells

Mouse	Cell line	^{125}I (c.p.m. bound per 10^6 cells)		
		EGF	gp70	Con A
	BALB/3T3 untransformed	6,700	2,040	59,500
	MuLV infected	7,400	40	NT
	Sarcoma virus-transformed, virus-producer	170	30	NT
	Sarcoma virus-transformed, non-producer	140	1,910	23,900
	SV40 transformed	5,900	1,820	14,400
Rat	NRK untransformed	7,600	NT	36,900
	Sarcoma virus-transformed, non-producer	60	NT	59,200
Mink	Mink lung untransformed	28,200	NT	64,200
	Sarcoma virus-transformed non-producer	1,500	NT	36,400

The binding assays were performed as in Fig. 1. All proteins were bound to monolayers of log phase cells containing approximately 1×10^6 cells per 60-mm Petri dish. The bindings were performed in binding buffer for 40 min at 37°C . The quantitative differences for the individual ligands used are as follows: mouse EGF, 3 ng of ^{125}I -labelled peptide containing approximately 110,000 c.p.m. and 10 μg of unlabelled material to correct for nonspecific binding; gp70, 6 ng of radiolabelled material containing approximately 68,000 c.p.m. and 10 μg of unlabelled peptide to correct for nonspecific binding; concanavalin A, 10 ng of radiolabelled peptide containing approximately 640,000 c.p.m. and 14.5 mg of alpha methyl-D-mannoside⁴² to correct for nonspecific binding. Of the cell lines tested the SV40-transformed clone (SV-A31-CL6) has consistently given somewhat lower levels of concanavalin A binding compared with both normal and other transformed clones. Whether this is a general property of SV40-transformed clones is not yet clear.

cell lines, it seems that there is one major class of receptors that bind EGF and there is little evidence for cooperative binding or for the existence of a significant fraction of secondary, low-affinity receptors.

Specificity of the cell membrane change

To determine whether the profound alteration in the EGF-binding properties of sarcoma virus-transformed cells is a manifestation of a more generalised membrane alteration, we tested other ligands that bind to cells (Table 3). The major mouse leukaemia virus glycoprotein, gp70, has high-affinity receptors on mouse cell membranes⁴¹. Normal mouse cells in culture can bind gp70, whereas cells that are actively producing type C viruses, either as a result of external infection or as a result of activation of endogenous virogenes, lose the ability to bind the purified viral envelope glycoprotein, because the receptors are occupied by gp70 molecules⁴¹. As shown in Table 3, clone A31 cells have detectable receptors for gp70 whereas cells infected with and producing mouse leukaemia viruses have none. Although sarcoma virus-transformed non-producer cells as well as transformed virus-producing cells do not bind ^{125}I -EGF, only those clones actually producing virus have lost detectable receptors for gp70. SV40-transformation affects neither the binding of gp70 to cell membranes nor the binding of EGF to its specific receptors.

Concanavalin A (con A), binds to cell membranes of

normal and transformed cells⁴². As shown in Table 3, there are only small differences between normal, MSV-transformed, or SV40-transformed cells in the binding of ^{125}I -labelled con A to cell membranes when low concentrations (7 ng ml^{-1}) are used. Insulin receptors have also been reported to show no difference in binding between MSV-transformed and SV40-transformed cells⁴³.

EGF binding after sarcoma virus infection

The loss of detectable EGF binding to MSV- and FeSV-transformed cells suggests that an alteration of EGF receptors is one of the specific effects of infection by some sarcoma viruses at least. However, since the cloned cell lines used in our studies were tested many generations after the initial transformation event, the results could reflect late, secondary effects associated with transformation but not necessarily with the sarcoma virus. To test this possibility, normal mink, rat and cat cells were infected with Kirsten sarcoma or feline sarcoma viruses and EGF binding was assayed as a function of time after infection. An effect on EGF binding was seen as early as 2 d after infection with feline sarcoma virus and 3 d after infection with murine sarcoma virus (Table 4). At this time, the cells were just beginning to show morphological evidence of transformation. By 6 d the effect on EGF receptors was pronounced, and by 2 weeks when most of the cells were clearly transformed, the EGF binding was greatly reduced. Since the

Table 4 Effect of MSV and FSV infection on the binding of EGF to the receptors of various mammalian cells

Cells	Virus	Time (d)	^{125}I -EGF (c.p.m. per 10^6 cells)		
			Control	MSV infected	% of control
MvILu	K-MSV (M28 baboon virus helper)	0	25,800	26,400	102.3
		3	29,400	19,800	67.3
		5	22,800	9,900	43.3
		14	25,500	4,800	18.8
NRK	K-MSV (SSAV virus helper)	0	12,500	14,200	113.6
		3	15,500	7,300	47.1
		6	14,200	4,300	30.3
		14	12,700	700	5.5
Cat embryo (FFc60WF)	GA-FSV (FeLV virus helper)	0	2,400	2,300	95.8
		2	2,800	1,400	50.0
		4	2,400	1,200	50.0
		6	2,500	950	38.0
		14	2,200	320	14.5

At time zero 20% confluent Petri dishes were infected with 1 ml of banded sarcoma virus stock diluted 1:10 with DMEM containing 10% calf serum. The controls were parallel flasks infected in the same manner with helper leukaemia virus free of sarcoma virus. At the indicated time after infection, two dishes each were taken for binding assays as described in the legend to Table 1 using ^{125}I -mouse EGF (3 ng containing 110,000 c.p.m.).

Table 5 Mouse cell virogene expression and oncogenic expression; availability of receptor sites

Cell line	Virus production	gp70 receptor binding	Cell transformation	EGF receptor binding
Mouse embryo diploid	—	+	—	+
BALB/3T3	—	+	—	+
BALB/3T3—MuLV producer	+	—	—	+
BALB/3T3—MSV-transformed, non-producer	—	+	+	—
BALB/3T3—MSV-transformed, MuLV producer	+	—	+	—

mammalian sarcoma viruses are unable to replicate by themselves⁴⁴, sarcoma virus preparations contain mixtures of defective sarcoma viruses and "helper" type C leukaemia viruses. Control cells used in these experiments, therefore, were infected with the helper virus alone (FeLV in the case of the cat cell infection, SSAV⁴⁵ for the rat cell experiment, and the baboon virus M28⁴⁶ for the mink cell infection). None of the non-transforming viruses could be shown to affect EGF binding. From these experiments we conclude that the alteration in EGF binding to MSV- and FeSV-transformed cells represents an early event following infection that can be demonstrated within two to three cell divisions; in fact, this change may serve as an early index of transformation by murine and feline sarcoma viruses.

EGF receptors and gp70 receptors

BALB/3T3 (clone A31) is not morphologically transformed and is not spontaneously producing endogenous type C viruses⁴⁷. It has 5×10^5 – 6×10^5 leukaemia virus gp70 receptors per cell⁴⁸ and 4×10^4 – 5×10^4 EGF receptors per cell (unpublished results). In these properties the clone resembles normal mouse diploid embryo cells (Table 5). After exogenous infection with mouse leukaemia viruses or after activation of endogenous type C viruses the cells no longer bind the viral envelope protein, gp70; they nevertheless continue to bind labelled EGF. Clones of A31 transformed by MSV but not producing virus, on the other hand, retain available receptors for gp70 but no longer bind labelled EGF. Cells that are both transformed by MSV and are producing virus have available receptors for neither gp70 nor for EGF.

EGF as a possible transforming protein

The genes which code for the gp70 receptor as well as those for gp70 itself are endogenous and genetically transmitted in mouse cells. In fact, gp70 has been shown to be a differentiation antigen found on the surface of normal mouse thymocytes^{47–49} and certain other cell types⁵⁰ which are not actively producing type C viruses. Similarly, both the genes for the EGF receptors and for EGF itself must be transmitted as part of the mouse genome. Our results suggest that an alteration in the EGF receptors is a significant early event associated with transformation by murine and feline sarcoma viruses. The decreased levels of EGF binding found in MSV-transformed cells might reflect (1) an indirect effect of the sarcoma gene product on the synthesis, degradation, or location of functional EGF receptors or (2) the production of endogenous EGF or a related substance that binds to the EGF receptors.

One possible model consistent with the data is that the sarcoma genes code for an EGF-related substance and, thus, inhibit binding by added hormone. Sarcoma viruses have arisen by recombination between "helper" type C viruses and sarcoma-specific cellular sequences^{6,9}. Thus, transformation resulting from expression of "sarc" genes need not be a consequence of sarcoma virus infection, but alternatively, may result from the expression of endogenous "sarc" genes (oncogenes). If the sarcoma gene expression is causally related to the alterations in EGF receptors, the model would predict that certain "non-viral" transformants would also show decreased levels of EGF binding. We have

so far examined 15 chemically-transformed clones derived from BALB/3T3 and have found one benzopyrene-transformed A31 clone that has greatly reduced EGF binding, similar to that seen with the MSV transformants. In this chemically-transformed line, it may be that an endogenous factor, perhaps EGF itself, is produced which can alter the EGF receptor and thereby provide a continued endogenous stimulus for cell division.

Various other polypeptide growth stimulatory hormones such as fibroblast growth factor (FGF)^{51,52}, nerve growth factor (NGF)⁵³, insulin⁵⁴ and multiplication stimulation activity (MSA)^{54,56} have been described. These also have some of the properties we might expect of a transforming protein. The specific receptors for each of these other hormones are distinct from those that interact with EGF^{57,58–60} and comparisons of DNA virus- and RNA virus-transformed cells have so far only been made for insulin receptors⁴³ where no differences were seen. The EGF binding data described here lead to the conclusion that SV40 and polyoma, each of which also carries a gene(s) for transforming proteins, exert their action elsewhere, and do not involve the EGF receptor-growth regulatory system.

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Partial denaturation mapping of cloned histone DNA from the sea urchin *Psammechinus miliaris*

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A cloned 5.6-kb DNA repeat unit of Psammechinus miliaris containing all five histone coding sequences of known order and polarity has been shown to fall clearly into a low melting and a high melting half by thermal denaturation. The topologies of the DNA sequences thus defined were determined by partial denaturation mapping in the electron microscope to gain further insight into the distribution of spacers and genes within histone DNA.

THE five histone coding sequences of the sea urchin *Psammechinus miliaris* alternate with spacer DNA within a (nominally) 6-kilobase (kb) DNA unit¹ which is tandemly repeated several hundred times^{2,3}. The coding sequences are on the same DNA strand⁴ and are arranged in the order H4, H2B, H3, H2A and H1 (ref. 1). Transcription proceeds in the H4 to H1 direction⁴. In sea urchins, neither the site(s) of initiation of transcription nor the nature of the primary transcript is known, but there is some evidence that HeLa cells contain a high molecular weight nuclear precursor to cytoplasmic histone RNA (M. Melli, G. Spinelli, H. Wyssling and E. Arnold, in preparation).

There has already been extensive restriction mapping¹ and some sequencing (unpublished results) of the histone DNA repeat units of *Psammechinus miliaris*. An alternative approach for the elucidation of the sequence organisation of this DNA is to examine partially denatured DNA molecules in the electron microscope. This will reveal the gross topologies of DNA sequences and will permit the identification of regions of particular interest within the molecule. We have taken this approach and have used for a protein-free spreading technique⁵, the first time that it has been applied to partial denaturation mapping in a quantitative way.

GC-rich genes and AT-rich spacers

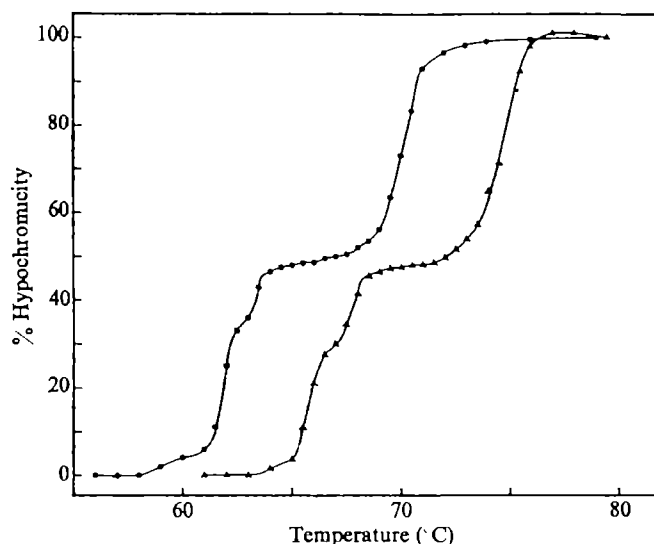
Histone mRNA of sea urchin has been found to be rich in GC (50-56%) by base composition analysis^{6,7}. RNA-DNA hybrids from the histone mRNA subfractions I-III and V of *Psammechinus*, now known to represent the individual mRNAs for H4, H2b, a mixture of H3 and H2A and for H1⁸, respectively, all have the high melting temperature of 75-76 °C in 0.1 × SSC^{2,3}, as would be expected from the high GC content of the sea urchin histone mRNA. By contrast, the intervening spacer DNA is rich in AT. This was first inferred from the banding position (1.704 g ml⁻³; ref. 9) of histone DNA in CsCl and was later substantiated by *T_m* measurements on isolated histone DNA¹. Consequently, histone

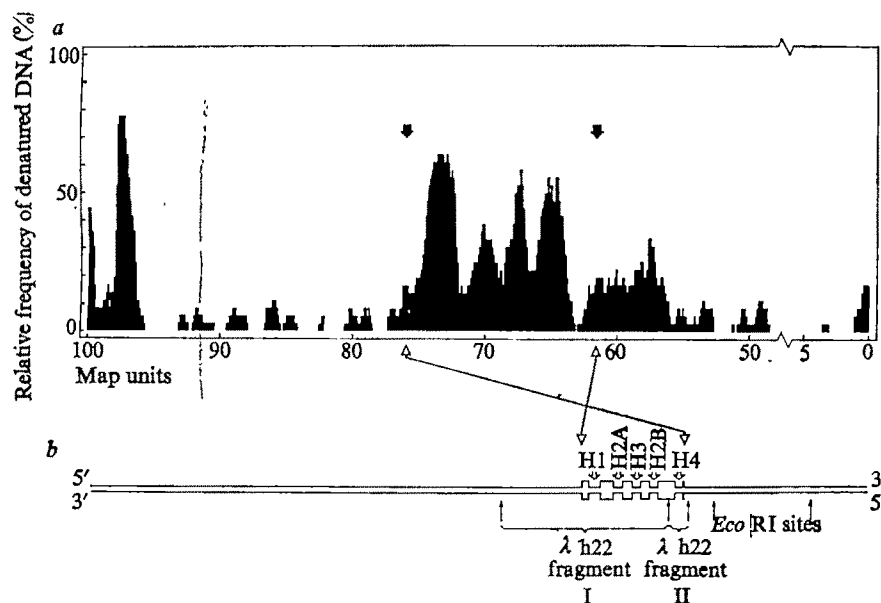
DNA consists of two major types of DNA—GC-rich histone coding and AT-rich spacer DNA sequences.

There are also some definite predictions on the amounts of GC-rich DNA that can be made. The coding sequences for the five histone polypeptides can be calculated to require about 2.13 kb (Table 1). Sea urchin mRNA H4, however, contains additional, untranslated sequences of about 100 nucleotides¹⁰. If stretches of similar size are also found in the other histone messengers, the coding sequences for all five would be of the order of 2.7 kb, or about half the repeat length, now determined to be 5.59 ± 0.35 kb by electron microscopy (see below).

The melting profile of the histone DNA, reclaimed from the recombinant phage λ b538 redB113 *imm*^{4,34} (Pm histone)-22 (ref. 11) by restriction⁴ is quite simple and has all predicted characteristics (Fig. 1). It falls clearly into early melting and late melting halves. The second major transition has a *T_m* of 74.8 °C (52% GC).

Fig. 1 Melting profile of reclaimed h22 DNA. h22 was isolated from λ h22¹¹ by restriction with *Hind*III and preparative gel electrophoresis as described earlier^{4,20}. The DNA was dialysed exhaustively against 0.1 SSC (▲) or 10 mM NaAc (●), pH 8, and the DNA (10 μ g ml⁻¹) was heated in a Beckman Acta III spectrophotometer. The heating increment was 0.5 °C per min. Bacterial DNAs from *Escherichia coli* and *Micococcus lysodeikticus* melted at 74 °C and 85 °C, respectively, histone mRNA/DNA hybrids at 75 °C (ref. 3).





Restriction of the recombinant DNA yields λ h22 fragments I and II of 9.1 and 1.1 kb. Others clones, with histone DNA inserted in the opposite way, produce fragments of 4.9 and 5.3 kb (unpublished results of W. Schaffner).

Fig. 2 a, Histogram of 37 partially denatured λ h22 molecules. The frequency of DNA denaturation with map units is given at a resolution of 50 base pairs or 0.12 map units. The areas of the black fields can be taken as a measure of the amount of denatured DNA. In the orientation chosen in Fig. 2a the transcription of the histone genes in the sea urchin would be left to right. The 10-kb 'right' arm of the receptor phage extends from map units 75.8–100%. It includes the typical λ blister¹⁶ at 97.2%. The insertion points for the eukaryotic DNA map at 61.5 and 75.8%, respectively, with a histone DNA intervening between them. On average 37% of the inserted h22 was denatured with blisters appearing at 65.0, 67.5, 70.0 and 73.3%. Even the terminal 80 base pairs near the H4 gene, seen as small fork in Fig. 4, can be detected as blister in intact recombinant DNA in some molecules. **b**, Restriction map of λ h22. The recombinant DNA is shown schematically in the orientation which is standard for λ phage. The large black arrows mark the integration points for h22 DNA which are also the cleavage sites for the *Hind*III restriction enzyme. The arrangement and polarity of the histone-coding sequences within the recombinant has been indicated (see text). There are four recognition sequences for the *Eco*RI restriction enzyme in the λ receptor phage, one in h22.

Its thermostability is that anticipated from the base composition of histone DNA and from the T_m of the mRNA–DNA hybrids. The fraction of DNA in this component is consistent with the idea that half the histone DNA is contributed by the coding sequences for the five histone mRNAs, inclusive of untranslated leading and trailing sequences. The other half of DNA melts at 65.6 and 68 °C, respectively, and because of its calculated composition^{12,13} of 34% and 40% GC would not be expected to code for histone mRNA¹. It is taken to represent the AT-rich spacer DNA.

Since the two major melting steps are clearly separated from one another by a pronounced plateau which extends over 4–5 °C, a situation prevails which is ideal for partial denaturation mapping. Conditions can be chosen in which all the AT-rich DNA segments are melted out while leaving the GC-rich regions virtually untouched. The topologies of the DNA segments can then be determined in the electron microscope.

Orientation of the recombinant histone DNA

Partial denaturation mapping in the electron microscope has the disadvantage that it is often impossible to establish the orientation of the gene arrangement from the blister map alone. But 'left' or 'right' of the molecule can be determined if the experiment is performed on uncleaved λ phage DNA into which histone DNA has been inserted in a unique orientation.

The recombinant DNA λ b538 *redB*113 *imm*⁴³⁴ (Pm histone)-22, λ h22 for short, is composed of three elements: the 27-kb left arm, the 10-kb right arm of the receptor phage and the 5.6-kb histone DNA unit intervening between them¹¹. Whenever required, the 5.6-kb histone DNA can be reclaimed from the recombinant DNA by digestion with *Hind*III and fractionation of the three fragments by gel electrophoresis⁴.

The λ -receptor phage contains, in addition to the single *Hind*III site into which the histone DNA was initially inserted¹¹, recognition sequences for a second restriction enzyme, *Eco*RI as indicated in Fig. 2¹⁴. The same enzyme also cleaves histone DNA asymmetrically within the spacer between the H4 and the H2B coding sequence (ref. 1, see Figs 2b and 4b). When the histone DNA recombinant is subjected to *Eco*RI restriction, the products will include two unique DNA fragments which are part histone and part λ DNA. The distances between the *Eco*RI cleavage site within histone DNA and the nearest sites in the λ molecule on either side will clearly depend on the orientation of the histone DNA molecule within the receptor phage. For λ h22, two fragments of 9.1 and 1.1 kb were found by the Southern hybridisation technique (ref. 15; data not shown). For this hybrid phage the orientation given in Fig. 2b applies.

Denaturation mapping requires heating of the DNA in low ionic strength media, to which formaldehyde is added to prevent snapback of the single-stranded DNA regions. For reclaimed histone DNA in 10 mM NaAc, pH 8, the plateau between low and high melting DNA was found at 60–65 °C (Fig. 1). The effects of formaldehyde cannot be gauged accurately because this agent fixes single-strand DNA regions during thermal breathing of the molecules¹⁶. Using the melting profiles as rough guidelines the conditions appropriate for partial denaturation of the histone DNA molecule had to be determined empirically. In the event, recombinant DNA in 10 mM NaAc, 3.5% formaldehyde, pH 8, was reacted at 61 °C for 10 min, the DNA spread with a protein-free technique (ref. 5; see legend to Fig. 3) and photographed in the electron microscope.

All intact molecules were selected which had the well known, characteristic blister in the distal area of the λ right arm^{16,17} (at map position 97.2%, see Fig. 2a) and the molecules were oriented

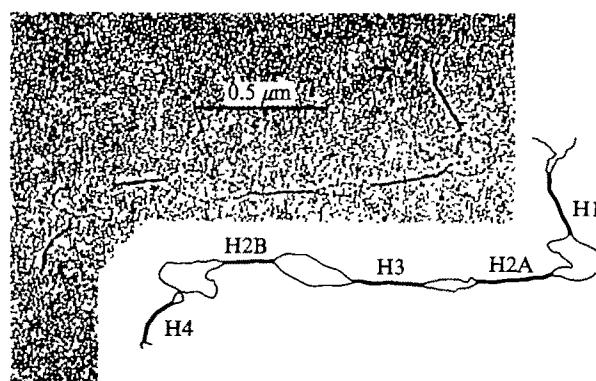
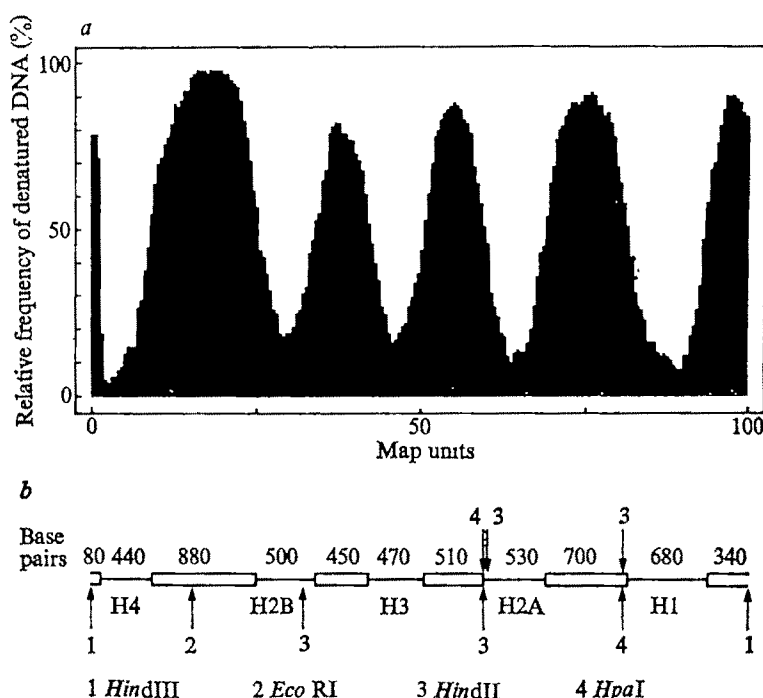


Fig. 3 Partially denatured h22 DNA. For explanation see text. h22 ($10 \mu\text{g ml}^{-1}$) was heated in 10 mM NaAc, 3.5% formaldehyde, pH 8, to 61 °C for 10 min, and cooled rapidly on ice. After 5 min an equal volume of a mixture containing 60% formamide, 180 mM NaAc, pH 8, and $7.2 \times 10^{-3}\%$ benzylidimethyl-alkylammonium chloride (BAC) was added. 2 μl of the DNA solution was deposited slowly onto the water surface in the centre of a Petri dish (diameter 16 cm) and the spreading of the film monitored by observation of the movement of fine graphite powder placed previously on the water. Carbon-coated 400-mesh copper grids were touched to the surface, the grids washed by floating them on bidistilled water, then stained in uranyl acetate and shadowed with Pt-C. The spreading technique was essentially that of Vollenweider *et al.*⁵ Electron microscopic pictures on Kodak image plates were taken at an instrumental magnification of $\times 8,500$ in a Siemens Elmiskop 101 and the topologies of the DNA segments were determined²⁶ using a map ruler at 1 mm resolution.

Fig. 4a. Histogram of partially denatured h22. The diagram depicts the frequency of denatured DNA with map distance calculated from 118 partially denatured h22 molecules. The orientation of the DNA has been chosen on the basis of experiments described in Fig. 2a and b. On average, 53% of the h22 DNA had been denatured as was required on the basis of the melting profile shown in Fig. 1b. Assignments of mRNA coding, spacer and restriction enzyme sequences to the partial denaturation map. The distribution and the average length of DNA in blisters and double-stranded DNA was calculated from the histogram shown in (a). The length of denatured DNA segments were shortened by a factor of 1.1 to correct for the more extensive stretching of single-stranded DNA. The overall length of the molecule was taken at 5.6 kb (see text). The assignment of the coding sequences was made on the basis of published hybridisation data¹. The known cleavage sites of a selection of restriction enzymes^{1,20} have been indicated in the map. 1 = *Hind*III, 2 = *Eco*RI, 3 = *Hpa*I, 4 = *Hind*II. Transcription of the coding sequences is left to right⁴.



with respect to this region. Thirty-seven partially denatured λ h22 molecules were standardised to a hundred map units and the frequency of occurrence of all blisters along the λ DNA molecules were determined. The diagram in Fig. 2a was thus obtained.

Partial denaturation map of histone DNA

First, reclaimed histone DNA was spread for inspection in the electron microscope⁵ and the lengths of the native molecules determined by comparison with added T7 marker molecules. Thirty-five T7 molecules, taken to represent 37.8 kb (ref. 18), averaged out at $12.64 \pm 0.56 \mu\text{m}$. 235 h22 molecules were measured from the same grids. A length of $1.87 \pm 0.12 \mu\text{m}$ or $5.59 \pm 0.35 \text{ kb}$ was found.

With the aim of achieving denaturation of approximately 50% of the DNA molecule, h22 was heated to 61–62.5 °C in 10 mM NaAc, 3.5% formaldehyde, pH 8, and spread for electron microscopy. Partially denatured histone DNA molecules like that shown in Fig. 3 were obtained. The h22 molecule terminates in a small fork at one end, a larger one at the other. Because of this, the denaturation pattern is clearly asymmetrical and so there was no difficulty in orientating the DNA molecules. The denatured and native lengths of 118 molecules in all were measured and their overall lengths were standardised to 100 map units. The frequency of denatured DNA stretches in these molecules is given as a function of map distance at a resolution of 28 base pairs in Fig. 4a.

Anatomy of the cloned histone DNA

The histogram in Fig. 4a shows that the *Psammechinus miliaris* histone DNA h22 consists of AT- and GC-rich DNA stretches intermingled with one another. A small open fork and a small double-stranded stretch are separated, on the left, from a series of three native segments of similar size. Adjacent to these, further to the right, is a middle-sized blister followed by a relatively large unmelted region which terminates, in most cases, in an open fork structure. Since in the sea urchin the 5.6-kb repeat units are joined together without any intervening DNA¹⁹ and are tandemly arranged many hundred times². The two terminal forks can be imagined to be linked in such a way that on denaturation they would form an intact fifth blister on chromosomal DNA.

Except for the joining points between λ and inserted histone DNA, marked by arrows, the blister map of the integrated and the reclaimed isolated h22 DNA shows striking similarities. The black areas in the histogram of Figs 2a and 4a contributed by denatured DNA are clearly different from one another and if numbered 1–4,

from left to right, can be arranged in the order $2 \leq 3 < 4 < 1$ in both diagrams. The polarity of the histone gene cluster in the λ -receptor phage is known (see above). It can be applied to the isolated h22 molecule on the basis of the distinctive blister pattern for both these kinds of molecules. This has been done in Figs 3c and 4b.

For quantitative considerations it is more convenient to discuss the DNA lengths in base pairs rather than in map distances. For this, we have to take account of the fact that denatured DNA⁵ is stretched out by a factor of 1.1 compared with native DNA and hence that the length would be overestimated in an uncorrected linear diagram. The standardised length in base pairs, calculated from a line dissecting the diagram of Fig. 4a at half height, is given in Fig. 4b. The summation of the lengths of all double stranded DNA segments (Table 1) shows that, on average, 53% of each molecule had been denatured.

The partial denaturation map supports the general model for *Psammechinus* histone DNA in which the five genes coding for the five histone proteins alternate with AT-rich spacer DNA. A similar arrangement has been made visible in the electron microscope for histone DNA from another sea urchin by M. Wu, D. Holmes and N. Davidson (California Institute of Technology) using their novel RNA–DNA hybridisation procedure. An intermingling of spacers and genes had previously been suggested by the results of a combined attack on the histone DNA by restriction and molecular RNA–DNA hybridisation¹. In these experiments, it was possible

Table 1 Number of base pairs in histone and histone mRNA coding sequences

Histones	No. of amino acids in protein	Amino acid coding sequence (bp)	mRNA coding sequence (bp)	GC-rich DNA segments (bp)
H4	102 (refs 21 and 22)	306	400 (ref. 8)	440
H2B	125 (ref. 23)	375	ND	500
H3	135 (ref. 24)	405	ND	470
H2A	128 (ref. 24)	384	ND	530
H1	Heterogeneous approx. 220	660*	ND	680
all five		2.62 kb		2.13 kb

Values for H3, H2A and H1 are those for mammals

*There is some circumstantial evidence²⁵ that the H1 of the sea urchin may be smaller than that of mammals

to subdivide the histone DNA repeat unit into five segments of similar size, each of which hybridised to just one histone mRNA but contained a considerably greater amount of DNA than would be required to code for a single histone protein.

In that work, the restriction enzymes *HindIII*, *HindII*, *EcoRI* and *HpaI* were shown to cleave histone DNA between the coding sequences, that is in spacer DNA^{1,20}. Since these cleavage sites have all been mapped by gel electrophoresis of the restriction fragments, their positions can now be found on our partial denaturation map (Fig. 4b). The cleavage sites fall into the blister regions or just within the GC-rich DNA segment near the interphase between melted and unmelted DNA with the exception of one *HindII* site which lies near amino acid 105 of the H2B coding sequence (unpublished results). It has been found that other restriction enzymes cut preferentially in coding sequences, while leaving spacer DNA virtually intact²⁰.

The sequence of 97 nucleotides from the 'left' end of h22 has been determined (unpublished results of H. O. Smith and M. L. Birnstiel). The first 60 of these averaged at a GC content of 34%, while the subsequent DNA stretch contained 50% GC. This demonstrates that the small fork detected in our partial denaturation map near the H4 coding sequence did not arise simply due to breathing of the DNA ends, but reflects the compositional bias of this terminal DNA segment. Here too, there is good accordance between the biochemical and the structural data. Throughout the work we have also noted that the protein-free spreading technique⁵ has provided a reliable means for partial denaturation mapping at high resolution.

Perhaps the most striking correlation that can be drawn is that between the molecular weight of histone proteins and the size of the GC-rich DNA segments delimited on either side by the DNA blisters. The H4 proteins of sea urchins is known to be made up from 102 amino acids^{21,22} and its mRNA to contain some 400 bases^{6,7}. In our map, the DNA assigned to this mRNA is 440 base pairs (Table 1). The largest histone, H1, contains some 220 amino acids²³ and hence some 660 base pairs would be required for its

coding. In keeping with this, the largest double-stranded region is found at the location predicted for the H1 gene. Here, as well as for the middle group of the histone-coding sequences H2A, H2B and H3 there is an excess of base pairs which may code for the leading and trailing sequences of these histone mRNAs. The base sequence arrangement of some of these regions, of spacers and genes will be reported shortly.

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Overlapping genes in bacteriophage ϕ X174

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Bacteriophage ϕ X174 genes D and E are translated from the same DNA sequence but in different reading frames.

BACTERIOPHAGE ϕ X174 contains a single strand of DNA approximately 5,400 nucleotides in length, which has been shown to be of the same sense as the mRNA and to code for nine cistron products. As shown in Table 1, there is an apparent discrepancy between the length of the DNA in the genome and the amount required to code separately for the proteins; significantly more DNA would be needed to code for proteins of the accepted molecular weights than is available. An almost complete sequence of ϕ X DNA has been obtained in this laboratory using the "plus and minus" technique of Sanger and Coulson¹, confirming that the length of the DNA is not more than 5,400 nucleotides (G. M. A., B. G. B., N. L. Brown, A. R. Coulson, J. C. Fiddes, C. A. H., F. Sanger, P. M. Slocombe and M. Smith, in preparation). At the same time, a study of the amino acid sequences of the major ϕ X coded proteins has enabled the boundaries of genes D, J, F, G and H to be located on the restriction map of ϕ X DNA (Fig. 1). Table 1 shows that the molecular weights of the F, G, J and H proteins have been found to be close to correct, while the

D protein molecular weight is somewhat larger than the sodium dodecyl sulphate (SDS) gel estimate. It has not yet been possible to determine directly the boundaries of genes A, B and C in the remaining region between gene H and gene D. The order of genes D to H is in agreement with the genetic map of ϕ X reported by Benbow *et al.*⁴ except that they located gene E between D and J. Here we report the DNA sequence from the promoter preceding gene D through to gene J. We find that gene E amber mutations lie within the DNA sequence which codes for the gene D protein, and interpret this to mean that genes D and E overlap on the DNA sequence. Sequences of gene E amber mutations show that D and E are translated in two reading frames from a common DNA sequence.

It should be emphasised that genes D and E seem perfectly independent by normal genetic criteria. The D protein is produced in large amounts in the infected cell. It is necessary for the production of viral single-stranded DNA, but its precise role in this process is unclear and there is no evidence that it is directly involved in DNA synthesis^{5,6}. Gene E is responsible for lysis of the host cell. All nonsense mutants in gene E characterised so far produce completely functional full size gene D protein. In fact, the protein chemistry of the gene D protein which is reported in this paper has been carried out using material produced by an amber mutant in gene E (*am3*). Similarly, normal gene E function has been observed in the gene D nonsense mutants.

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Table 1 ϕ X174 coding capacity

Gene	Protein molecular weight from SDS gels*	Number of nucleotides From protein molecular weight estimates	From nucleotide sequence	Protein molecular weight from sequence information†
A	62,000	1,701	>1,851	67,400
(A')	35,000	(960)		
B	19,000–25,000	>522		
C	7,000	192		
D	14,500	399	456	16,811‡
E	10,000–17,500	>273	(273)	9,940
J	5,000	138	114	4,097‡
F	48,000	1,317	1,287	46,700
G	19,000	522	525	19,053‡
H	37,000	1,014	981	35,500
Total		>6,078	5,374§	
Length of ϕ X174 DNA		~5,370		

*There is considerable variation in reported molecular weight estimates of ϕ X174 coded proteins. The values given are those most frequently recorded, or recently reinvestigated^{4,22,28–33}.

†Protein molecular weights are calculated from the number of nucleotides using the formula:

$$\text{Protein molecular weight} = \text{No. of nucleotides} / (3 \times 0.00915)$$

‡These values are calculated from the amino acid sequences (Freymeyer *et al.*, in preparation; ref. 35 and this paper).

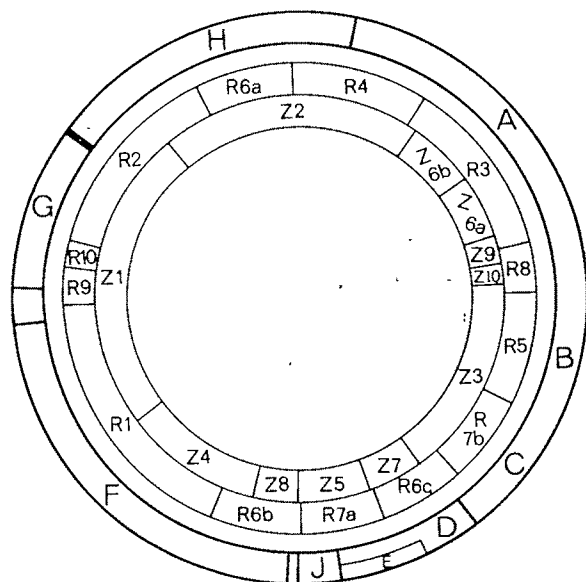
§This figure includes the 160 nucleotides of the intercistronic regions so far determined.

Nucleotide sequence analysis of the gene D region

The cleavage sites of ten restriction enzymes of different specificities have been mapped on ϕ X174 RF I (double stranded) DNA. The cleavage sites of these enzymes in the gene D region are shown in Fig. 2.

Using the restriction fragments shown in Fig. 2 as primers with either the (+) or (–) strands of ϕ X DNA as a template, sequences of approximately 100 residues on either side of all the restriction enzyme sites in this region were determined using the plus and minus technique of Sanger and Coulson¹. Figure 3 shows a good example of this method using fragment *Taq* 4 as a primer on ϕ X (–) strand DNA cleaved with *Mbo*II. A long and a short fractionation of the same reactions is shown, from which an almost unambiguous sequence of at least 126 residues can be deduced, starting 14 residues from the *Mbo*II 1/7 site.

Fig. 1 A map of ϕ X174 DNA. The boundaries of genes D, E, J, F, G and H are shown aligned with the restriction maps of *Hae*III (fragments designated Z) and *Hind*II (fragments designated R)^{2,3}. The precise location of genes A, A', B and C have not yet been determined.



In this way a large number of overlapping sequences from each restriction site and from both strands were obtained which was more than enough to deduce the entire sequence of the gene D region. Although the method is very reliable these overlapping sequences overcame some problems encountered with the plus and minus technique. One disadvantage is that the first 15 residues from the restriction site are not normally seen on the acrylamide gels. This is probably due to the instability of short products on the template which are subsequently degraded by the exonuclease activity of the DNA polymerase present during the restriction enzyme digest of the plus and minus reactions. Another problem encountered is unequal "jump sizes" on the gel between products differing by one residue in length. These occurred in specific regions of the sequence, probably due to local secondary structure at the end of products which were not completely denatured during gel electrophoresis. Electrophoresis at high current (high temperature) gives much improved separation. This problem could also be overcome by priming in the same region from an adjacent restriction site but on the complementary strand. An early difficulty in deducing accurately the number of residues in a run of a particular nucleotide has been overcome by including alongside the plus and minus reactions on the gel a digest of the initial incorporation. This gives a graticule of every possible chain length of the newly synthesised DNA from the restriction enzyme site.

In some cases other techniques such as limited exonuclease digestion of rCTP or rGTP substitution products^{9,10} and depurination of nick-translated restriction enzyme fragments¹¹ have been used to confirm the sequences deduced from the plus and minus system where they were in any doubt. The DNA sequence is shown in Fig. 4.

Amino acid sequence analysis of the D protein

A further confirmation of the DNA sequence was provided by amino acid sequence analysis of the gene D protein. Figure 5 shows the sequences of the protein and of the peptides derived by trypsin, chymotrypsin, thermolysin and cyanogen bromide digestion. The DNA sequence has been used for overlapping several peptides, and amino acids 58, 59, 93 and 123 were not identified in the protein work. The sequence of the first eight amino acids of this protein has been reported by Farber⁶ but it should be noted that our sequence differs by a serine replacing arginine at residue 7. The calculated molecular weight of the protein is 16,811 which is somewhat higher than the estimates from SDS gel electrophoresis of 14,500 daltons

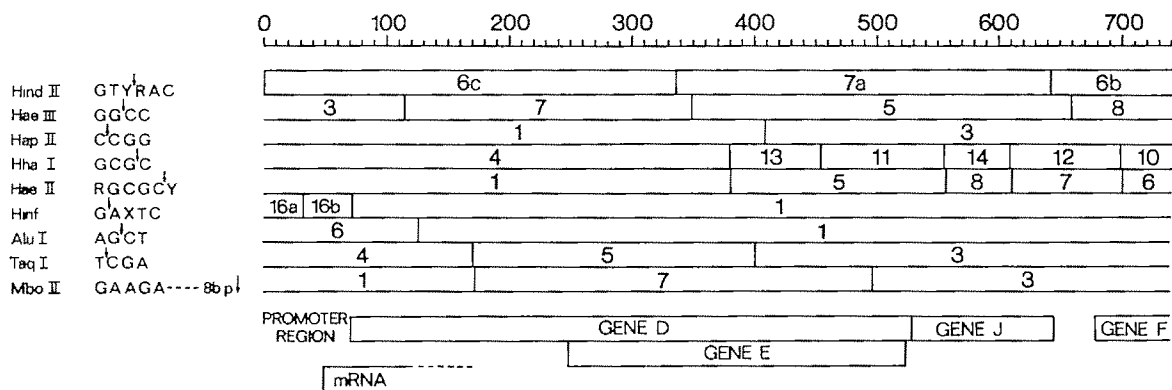


Fig. 2 Restriction map of the gene *D* region. The cleavage sites are shown of *Hind*II, *Hae*III (refs 2 and 3), *Hap*II (ref. 7), *Hha*I, *Hae*II, *Hinf*, *Alu*I (ref. 3), *Taq*I (S. Sato, C. A. H., and J. I. Harris, in preparation), *Mbo*II (N. L. Brown, C. A. H., and M. Smith, in preparation)

(Table 1). In 8 M urea-SDS gels the molecular weight estimate is more accurate at ~16,000 (G. M. A., unpublished).

Location of the promoter and genes *D* and *J*

The amino acid sequence analysis of the gene *D* product unequivocally defines the boundaries of gene *D* on the DNA sequence since termination codons are found in phase before the initiation codon and after the C-terminal amino acid codon. This rules out the possibility of a longer precursor to the gene *D* protein. The gene *D* initiation codon lies within the *Hind*II 6b-*Hae*III 3 region reported by Chen *et al.*¹² to contain an RNA polymerase binding site. Smith and Sinshemer¹³ have identified the *in vitro* mRNA start from this region as ppp(C) G-A-U-G-C. We find that this sequence occurs 30 residues to the left of the initiation codon of gene *D* (Fig. 4).

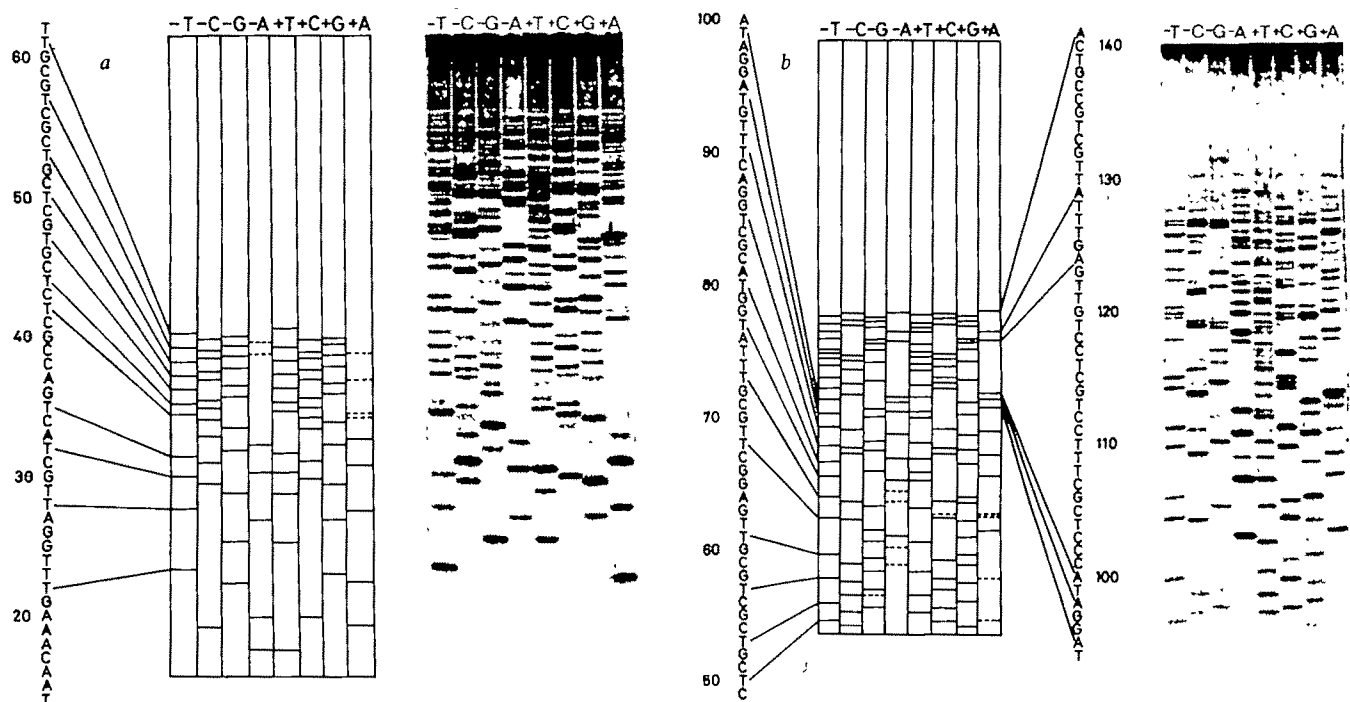
The termination codon of gene *D* overlaps by one nucleotide the initiation codon of gene *J* in the sequence T-A-A-T-G. Overlapping initiation and termination codons have also been

observed in *E. coli* tryptophan mRNA¹⁴. In this case the sequence was shown to be U-G-A-U-G.

Genetic mapping experiments of Benbow *et al.*⁴ suggested the existence of a gene *J* in this region coding for the smallest structural component of the virion. Alignment of the DNA sequence with the amino acid sequence of a small basic protein isolated from the virion (D. Freymeyer, P. R. Shank, T. Vanaman, C. A. H. and M. H. Edgell, in preparation) clearly demonstrates that it is coded by the gene immediately following *D*. We suggest that gene *J* be explicitly defined as the gene coding for this protein since marker rescue experiments show that the mutation (*am6*) which had genetically defined *J* (ref. 4) is located in *Hae*III fragment 7 (ref. 15) which ends 179 nucleotides before the initiation codon of *J* as defined here.

The complete nucleotide sequence of gene *J* has now been determined and has been shown to extend from the end of gene *D* to within approximately 35 nucleotides of the initiation codon of gene *F* (B. G. B., unpublished). This establishes the

Fig. 3 a, An autoradiograph of a plus and minus¹ sequencing gel using fragment *Taq*4 as a primer on ϕ X (—) strand template. The products were cleaved with *Mbo*II. A diagram showing the interpretation of the sequence is shown alongside. Electrophoresis was continued until the bromphenol blue marker reached the bottom of the gel. b, The same reaction as in a but fractionated for a longer time, such that the xylene cyanol marker migrated near to the bottom of the gel.



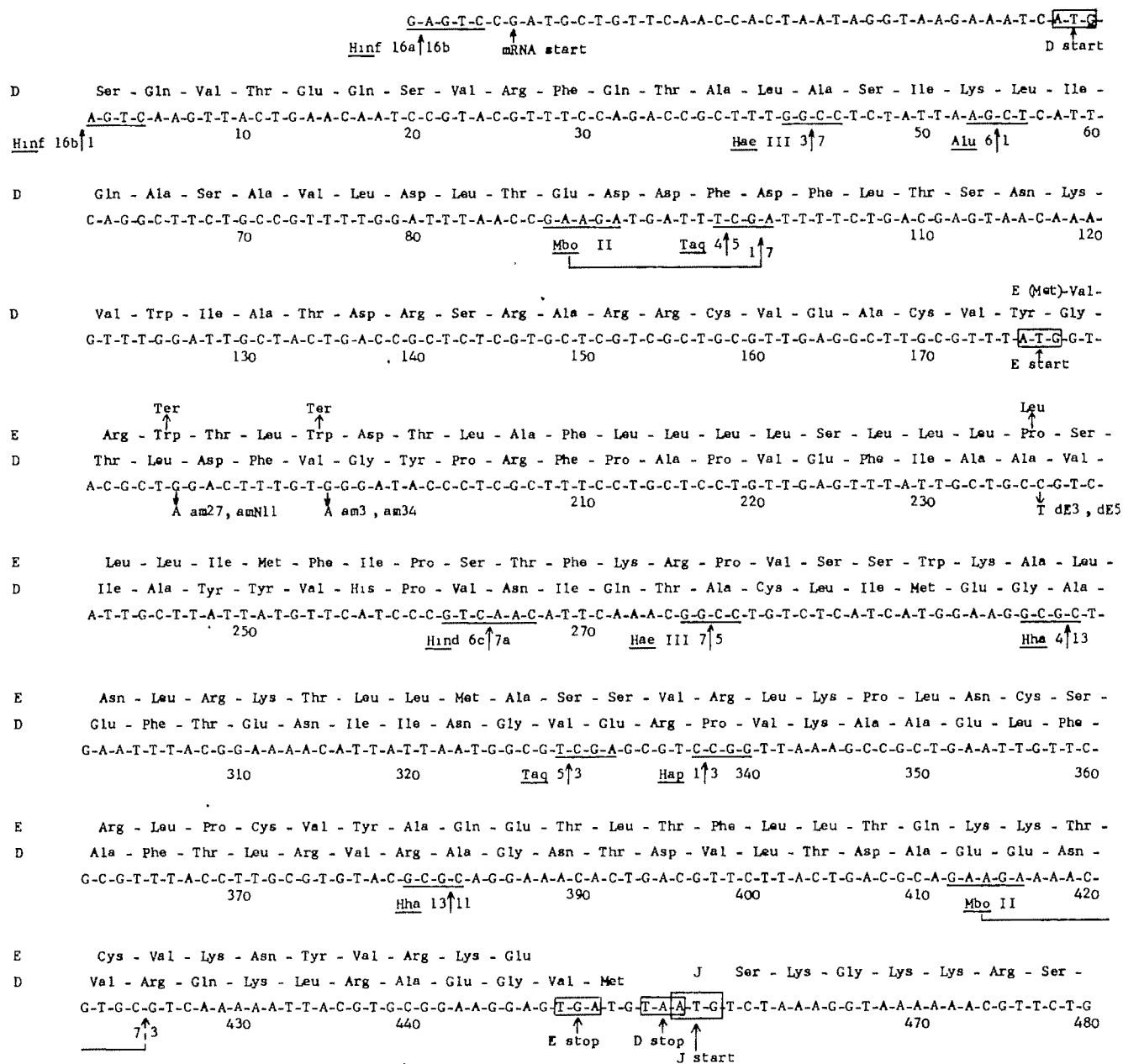


Fig 4. The nucleotide sequence of the gene *D* region. The amino acid sequences of the *D* protein (see Fig. 5) and the *J* protein are aligned with the DNA sequence. The amino acid sequence of the gene *E* product predicted from the DNA sequence is also shown.

order of genes *D*, *J* and *F* in this region, but gene *E* which mapped genetically immediately following gene *D* (ref. 4) is not accounted for.

Location of gene *E* mutants within gene *D*

It is possible to rescue genetic markers from restriction fragments¹⁶ and thus to establish the location of genetically defined sites on the physical map of ϕ X174. This technique has been applied to locate gene *E*. The double-stranded RF DNA of wild-type ϕ X174 was cut with restriction endonucleases and the resulting fragments were purified by gel electrophoresis. Fragments were denatured and annealed to the viral single-stranded DNA bearing a gene *E* mutation.

Spheroplasts of *E. coli* were transfected with the resulting complexes and the progeny phage were selectively assayed for wild-type plaque-forming particles. Wild-type marker can only be rescued from fragments which span the site of the mutation. Several gene *E* mutants were tested in this way, including *am* nonsense mutants. All were rescued by the wild-type *Hae*III fragment Z7 (Table 2). Weisbeek *et al.*¹⁵ have

also mapped one of these mutants (*am3*) and the mutant (*am6*) (probably a gene *E* mutant) in fragment Z7. Surprisingly, inspection of the nucleotide and amino acid sequence data showed that this fragment is completely contained within gene *D* (Figs 2 and 4). Since gene *E* *am* mutants make a full length *D* protein, these gene *E* nonsense codons cannot be in phase with the translation of gene *D*.

Nucleotide sequence of gene *E* mutants

Sequences within *Hae*III fragment Z7 have been determined for several ϕ X174 strains bearing mutations in gene *E*. Figure 6 compares a gel which displays the sequence synthesised on a viral strand template containing *am3* (gene *E*) with a gel showing the corresponding wild-type sequence. The mutation clearly produces a C→T change in the synthesised minus strand which corresponds to a G→A transition at position 195 in the viral (plus) strand sequence (Fig. 4). Thus the mutation results in the minus strand sequence 5'-C-T-A-3' which corresponds to the amber codon 5'-T-A-G-3' on the viral strand. The phase of this nonsense codon is displaced one nucleotide to the right

	5	10	15	20	25
Sequencer	Ser-Gln-Val-Thr-Glu-Gln-Ser-Val-Arg-Phe-Gln-Thr-Ala-Leu-Ala-Ser-Ile-Lys-Leu-Ile-Gln-Ala-Ser-Ala-Val-				
Tryptic peptides	Ser-Glx-Val-Thr-Glx-Glx-Ser-Val-Arg-Phe	Thr-Ala-Leu-Ala-Ser-Ile			
Chymotryptic peptides	Ser-Glx-Val-Thr-Glx-Glx-Ser-Val-Arg-Phe	Gln-Thr-Ala-Leu	Ala-Ser-Ile-Lys-Leu	Ile-Gln-Ala-Ser-Ala-Val-	
Thermolytic peptides	Ser-Glx-Val-Thr-Glx-Glx-Ser	Val-Arg	Ile-Lys-Leu	Ala-Val-	
	30	35	40	45	50
Sequencer	Leu-Asp-Leu-Thr-Glu-Asp-Asp-Phe-Asp-Phe-Leu-Thr-Ser-Asn-Lys-Val-Trp-Ile-Ala-Thr-Asp-Arg-Ser-Arg-Ala-				
Tryptic peptides					Ser-Arg-Ala-
Chymotryptic peptides	Leu-Asx-Leu-Thr-Glx-Asx-Asx-Phe(Asx, Phe, Leu)	Thr-Ser-Asn-Lys-Val-Trp	Ile-Ala-Thr-Asx		
Thermolytic peptides	Leu-Asx-Leu-Thr-Glx-Asx-Asx-Phe-Asx-Phe		Ile-Ala(Thr)Asx(Arg)Ser-Arg	Ala	
	55	60	65	70	75
Sequencer	Arg-Arg-Cys-Val-Glu-Ala-Cys-Val-Tyr-Gly-Thr-Leu-Asp-Phe-Val-Gly-Tyr-Pro-Arg-Phe-Pro-Ala-Pro-Val-Glu-				
Tryptic peptides	Arg	Arg			
Chymotryptic peptides		Gly-Thr-Leu-Asp-Phe	Val-Gly-Tyr-Pro-Arg-Phe-Pro-Ala-Pro-Val-Glu-		
Thermolytic peptides	Arg-Arg-Cys	Val-Glu-Ala-Cys			
	80	85	90	95	100
Sequencer	Phe-Ile-Ala-Ala-Val-Ile-Ala-Tyr-Tyr-Val-His-Pro-Val-Asn-Ile-Gln-Thr-Ala-Cys-Leu-Ile-Met-Glu-Gly-Ala-				
Tryptic peptides					
Chymotryptic peptides	Phe-Ile-Ala-Ala-Val-Ile-Ala-Tyr	Tyr	Val	Pro-Val-Asx-Ile-Glx-Thr	Cys-Leu-Ile(Mat)Glu-Gly-Ala-
Thermolytic peptides		Val-His-Pro			Leu-Ile-Met-Glu-Gly-Ala-
CNBr peptides					Glx-Gly-Ala-
	105	110	115	120	125
Sequencer	Glu-Phe-Thr-Glu-Asn-Ile-Ile-Asn-Gly-Val-Glu-Arg-Pro-Val-Lys-Ala-Ala-Glu-Leu-Phe-Ala-Phe-Thr-Leu-Arg-				
Tryptic peptides					
Chymotryptic peptides	Glu-Phe	Thr-Glx-Asx-Ile-Ile-Asx-Gly-Val-Glx-Arg-Pro-Val-Lys-Ala-Ala-Glx-Leu-Phe	Ala-Phe		
Thermolytic peptides	Glu	Phe-Thr-Glx-Asx	Ile-Ile-Asx-Gly-Val-Glx-Arg(Pro)	Val-Lys-Ala	Leu-Arg
CNBr peptides	Glx-Phe-Thr-Glx-Asx-Ile-Ile				
	130	135	140	145	150
Sequencer	Val-Arg-Ala-Gly-Asn-Thr-Asp-Val-Leu-Thr-Asp-Ala-Glu-Glu-Asn-Val-Arg-Gln-Lys-Leu-Arg-Ala-Glu-Gly-Val-Met				
Tryptic peptides	Val-Arg	Ala-Gly-Asx-Thr-Asx-Val-Leu-Thr-Asx-Ala-Glx-Glx(Asx)	Val-Arg	Gln-Lys-Leu-Arg	Ala-Glu-Gly-Val-Met
Chymotryptic peptides					Arg-Ala-Glu-Gly-Val-Met
Thermolytic peptides	Val-Arg-Ala-Gly-Asx-Thr-Asx-Val-Leu-Thr	Asx, Ala, Glx, Glx, Asx	Val-Arg-Gln-Lys		
CNBr peptides					
Carboxypeptidase					Gly-Val-Met

Fig. 5 Amino acid sequence analysis of the D protein. Sequences of ϕ X174 D protein and peptides derived by tryptic, chymotryptic, thermolytic and CNBr digestion. Solid bars indicate sites of cleavage; the broken bar shows partial cleavage. The residues shown were obtained by automatic (residues 1-17) or manual sequencing. Residues in parentheses were present in the amino acid composition but not identified in the sequencing work. Blanks indicate that the peptide was not sufficiently purified for a reliable amino acid analysis. The protein was isolated from 50 g of frozen ϕ X174-infected *E. coli* C (grown at the Microbiological Research Establishment, Porton Down). Cells were thawed with 50 ml buffer A (20 mM Tris, 10 mM $MgCl_2$, 1 mM mercaptoethanol, 1 mM EDTA pH 8.0), sonicated, and the cell debris centrifuged off, extracted with buffer A and 1 M NaCl, and centrifuged again. The supernatants were dialysed against buffer A at 4 °C, centrifuged again, and the supernatant loaded on a column (3 \times 20 cm) of Whatman DE-52 equilibrated with buffer A at 4 °C. After the unadsorbed proteins had been washed through a linear gradient was started (600 ml each A and A+0.6 M KCl) the D protein eluted about half way through the gradient as monitored by 15% acrylamide gel electrophoresis in 0.1% SDS.

Fractions containing the D protein were combined and 18.7 g $(NH_4)_2SO_4$ added per 100 ml at 4 °C. The resulting precipitate was centrifuged down, resuspended in buffer A, dialysed against buffer A and the suspension centrifuged again. The supernatant contained the D protein sufficiently pure for amino acid sequence analysis. It was dialysed extensively against water and freeze dried.

The amino acid sequencing was by the standard procedures as described previously^{34,35}. The amounts of *S*-carboxymethylated protein used were 2 mg for N-terminal automatic sequencing in the Beckman sequencer, 5 mg for succinylation and tryptic digestion, 2 mg for chymotryptic digestion, 15 mg for CNBr cleavage, and 5 mg for thermolytic digestion. Purification of the insoluble and the neutral thermolytic peptides was not attempted.

Table 2 Rescue of gene *E* mutants by restriction fragments from gene *D*

Gene <i>E</i> mutant	<i>Hae</i> III fragments			<i>Hha</i> I fragments		
	Z3	Z7	Z5	H8(ab)	H4	H13
<i>am3</i>	1.2×10^3	2×10^4	8×10^3	5×10^3	2×10^4	4×10^3
<i>am27</i>	1×10^3	$> 2 \times 10^4$	2×10^3	—	—	—
<i>am34</i>	6×10^1	$> 2 \times 10^4$	6×10^1	6×10^1	2×10^3	2×10^1
<i>amN11</i>	3×10^1	4×10^3	4×10^1	1×10^1	1.5×10^3	2×10^1
<i>dE3</i>	8×10^1	$> 2 \times 10^4$	6×10^1	4×10^1	1×10^4	4×10^1
<i>dE5</i>	1×10^1	2×10^3	$< 10^1$	$< 10^1$	1.2×10^3	$< 10^1$
S13 <i>amEn15</i>	2×10^3	4×10^3	2×10^3	—	—	—

Restriction fragments of wild-type RF DNA were purified by gel electrophoresis. Fragments were denatured, annealed to viral strand from each of the gene *E* mutants listed, and used to infect spheroplasts of *E. coli* as previously reported¹⁶. Wild-type plaque-forming phage were selectively assayed on *E. coli* C (*su*⁻). The number of wild-type phage per ml following chloroform lysis of the spheroplasts is tabulated, and positive results are underlined. In the experiment shown here only fragments from the gene *D* region were assayed. In another experiment (data not shown) all of the *Hae*III fragments and all of the *Hha*I fragments were assayed for the *am3* site and wild-type markers were only recovered from fragments Z7 and H4. The *am* mutants were assayed on *E. coli* HF4714. The *dE* mutants (see text) were assayed on MacConkey agar (Difco) plates. The phage sample was overlaid in a mixture of 2.5 ml of soft agar, 0.25 ml of 5 mg ml⁻¹ egg white lysozyme, 10 μ l of 1 M CaCl₂, and 0.3 ml of an overnight culture of *E. coli* C (*su*⁻). Plates were incubated at 37 °C.

of that used for translation of gene *D*. In this phase a tryptophan codon (T-G-G) is changed to *am* (T-A-G). The mutation does not produce a change in the D product since one valine codon (G-T-G) is changed to another (G-T-A). Similar analysis of an (*am3*) revertant showed that the wild-type sequence was present at position 195.

An independently isolated gene *E* mutant, *am34*, was shown to contain the same altered nucleotide sequence as *am3* (Fig. 4, data not shown). Two other independently isolated gene *E* amber mutants have also been sequenced (*am27* and *amN11*). Both result in G→A transitions on the viral strand at position 186 (Fig. 4, data not shown). This results again in a T-G-G→T-A-G change which produces an amber codon which is in phase with that found in *am3* and *am34*, and displaced one nucleotide to the right of the D translation phase. Again, no coding change is produced in the phase used for D translation (C-T-G→C-T-A, both coding for leucine). The sequence alterations produced by amber mutations at both these sites have been confirmed by pyrimidine tract analysis of the minus strand of *Hae*III fragment Z7. The G→A change reported here for *am* mutations induced by nitrous acid treatment of phage (plus strand) has been predicted by previous studies on the mechanism of nitrous acid mutagenesis^{17,18}.

One observation which seems inconsistent with the results presented here concerns the characterisation of a population of ϕ X174 deletion mutants¹⁹. These deletions were originally interpreted to be located in gene *E* and presumed to be defective only in lysis function. This is inconsistent with our results which predict that any gene *E* deletion would also have a defect in gene *D*. Further analysis of the ϕ X174 deletion population (R. L. Sinsheimer, personal communication) has shown, however, that it must contain deletions from all parts of the genome. We have also investigated this deletion population and obtained evidence that the population does not contain any particles whose only genetic defect is a gene *E* deletion. We observed that all gene *E* *am* mutants tested were able to form plaques efficiently on *su*⁻ hosts when plated on special medium containing bile salts and egg white lysozyme (see legend to Table 2). If simple gene *E* deletions exist it should be possible to clone them on such plates. In fact, about 1–2% of the particles in the deletion population do form plaques on these special plates but not on normal plates on *su*⁻ or *su*⁺ hosts. However, all 20 isolates tested reverted with frequencies reasonable for simple point mutations. Two of these isolates (*dE3* and *dE5*) were mapped by marker rescue on to Z7 and sequenced. Both isolates contain the same missense mutation which produces a predicted Pro → Leu (C-C-G → C-T-G) change in the gene *E* translation phase as defined by sequenced gene *E* amber mutations (position 237, Fig. 4). This would again produce an unchanged D protein since one alanine codon (G-C-C) is changed to another (G-C-T).

Extent of the gene *E* coding region

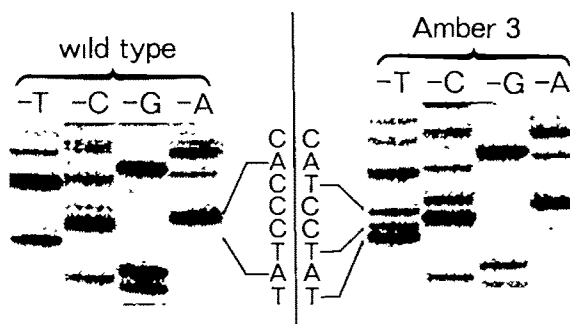
The gene *E* amber mutations are suppressible in *su*⁺ hosts indicating that gene *E* is being translated at those locations. It is therefore possible to determine the boundaries of the gene *E* coding region by looking for termination codons in the same phase as the amber codons. The termination codon at position 449–451 (Fig. 4) defines the 3' terminus of gene *E* protein as it is the first available termination codon in this phase after the amber mutations. Initiation of the gene *E* protein must occur between the termination codon at position 107–109 and the amber mutations. There are two possible initiation codons in this region, a G-T-G at position 146–148 and an A-T-G at position 176–178. Of these the A-T-G is the only initiation codon preceded by a sequence complementary to the 3' sequence of 16S rRNA that has been implicated in the initiation of protein synthesis^{20,21}. Figure 7 shows a comparison of the predicted gene *E* initiation site with other initiation regions found in ϕ X174. Starting at the A-T-G codon at residues 176–178 a polypeptide of 91 amino acids would be synthesised. We have been unable to identify clearly either the E protein or the *am3* fragment *in vivo* or *in vitro*, so amino acid sequence analysis has not been possible. However, the calculated molecular weight of the E protein is in good agreement with the identification of an *E* gene product with a molecular weight of 10,000 by Burgess and Denhardt²², although other estimates have varied (see Table 1).

Evolution of overlapping genes

The mechanism by which the gene *E* product acts to induce lysis of the infected cell is not clearly understood. There is no

Fig. 6 Sequence of the *am3* mutation site. *Hae*III fragment 5 was used as a primer with either wild-type or *am3* ϕ X (+) strand as a template. Only a portion of the autoradiograph of the minus system is shown in both cases with the sequences of the (–) strand deduced. The viral strand sequence is therefore:

wt 5'-G-T-G-G-G-A-T-A-3'
am3 5'-G-T-A-G-G-A-T-A-3'



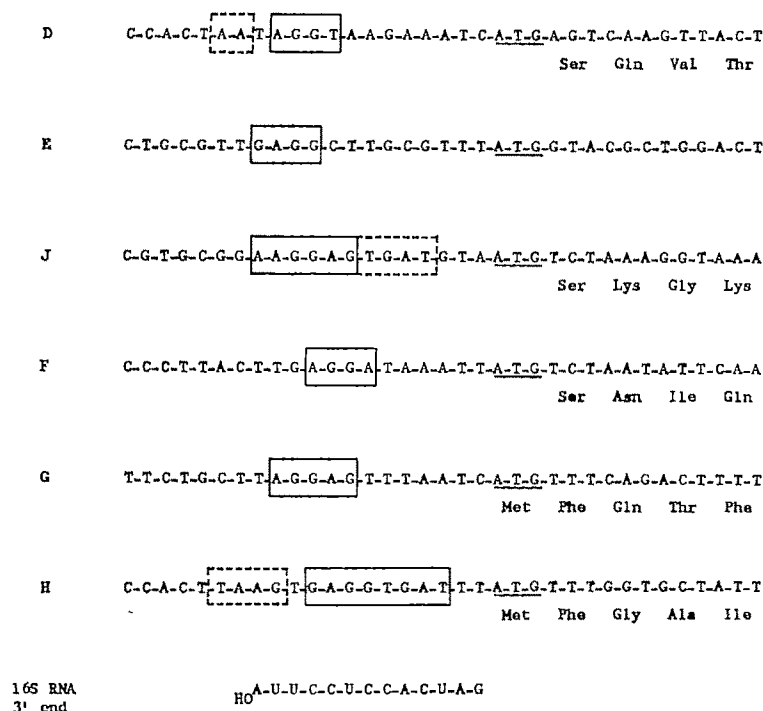


Fig. 7 Comparison of the predicted gene *E* initiation site with other known ϕ X174 initiation sites. The boxed areas indicate sequences complementary to the 3' end of 16S rRNA. Dashed boxes show further complementarity if some nucleotides are looped out or mismatched. Known N-terminal amino acid sequences are also shown.

evidence that it functions as a lysozyme. Bile salts in the presence of egg white lysozyme are able to induce lysis of cells infected with mutants deficient in gene *E* function, although lysozyme alone is not sufficient. This suggests that the gene *E* protein may act on the cell membrane. The predicted amino acid sequence of the *E* protein (Fig. 4) is very hydrophobic in the N-terminal region, in particular in the sequence Leu¹¹-Leu¹²-Leu¹³-Leu¹⁴-Ser¹⁵-Leu¹⁶-Leu¹⁷-Pro¹⁸-Ser¹⁹. A large percentage of hydrophobic amino acids including tracts of leucine residues have been observed at the N terminus of precursors to several pancreatic secretory proteins²³ and in the N-terminal region of the light chain IgG precursor²⁴. These N-terminal regions have been proposed to function in the transfer of the proteins across the microsomal membrane. The similarity between these sequences and the N-terminal region of the gene *E*-protein is consistent with a membrane-related activity.

An analysis of possible secondary structure in the *E* protein using the rules of Chou and Fasman²⁵ predicts that the N-terminal hydrophobic region could consist of an α -helix terminating before the proline at residue 21. The missense mutations dE3 and dE5 cause a Pro to Leu change at residue 21. This mutation, which causes a loss of lysis function, could affect the secondary structure of the *E* protein by preventing α -helix termination at this point.

It is interesting to speculate on the evolution of overlapping genes *D* and *E*. It is possible that the size of the ϕ X174 genome is limited by packaging or other unknown constraints. If so, the genes required could only be fitted in either by overlapping or by reading from both strands of the DNA. The gene *D* codons contain a high proportion (40%) of T residues in the third position, as is observed in other ϕ X174 genes^{26,27}. This suggests the possibility that gene *D* arose first and that ancestors of ϕ X174 could survive without a specific lysis gene in their natural environment. It is clear that if some mutational event led to initiation of protein synthesis in a phase one nucleotide to the right of the *D* reading phase the resulting codons would have a high proportion of T residues at the second position. Such codons specify hydrophobic amino acids and it seems plausible that a polypeptide could be produced which would interact with the host cell membrane. This could provide a basis for natural selection leading to an efficient lysis gene. It is worth noting that a gene *E* amber mutant in the closely related phage S13 is rescued by fragment Z7 from *wt* ϕ X174 (Table 2).

It seems likely that translation of the gene *D* protein, which is made in large amounts, would obstruct binding of ribosomes at the *E* initiation site. This might be a convenient control if the *E* protein is only required in small amounts. On the other hand, some control may be required to facilitate initiation of the *E* protein, for example, mRNA processing. Initiation of the gene *J* protein poses a similar problem, since the sequence involved in gene *J* ribosome binding is being translated in both *D* and *E* phases.

The fact that genes *D* and *E* overlap alleviates to some extent the problem of fitting the known ϕ X genes into the available DNA (Table 1). However, it is clear that if the molecular weight estimates of the A, B and C proteins are correct, they cannot be separately coded in the region between genes *H* and *D*. Thus, in spite of the severe constraints imposed on the amino acid sequence by translation in two phases, it seems possible that other examples of overlapping genes may be found.

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letters to nature

Determination of the time of the shell ejection in nova outbursts

THE light curve of novae is characterised by a sharp rise in brightness, followed by a decrease in light intensity at an ever slowing rate (see Fig. 1). The optical spectrum of novae near maximum consists of emission and absorption line systems superimposed on a continuum. One of the main features of the evolution of novae spectra is the fading of the continuum relative to the emission lines. In late stages, all novae develop the 'nebular' spectrum, consisting almost exclusively of emission lines with no continuum or with a very weak one (see ref. 1). The analysis of the light curve of a nova can reveal the time of ejection of its main shell. In four galactic novae this time was found to vary between 19 and 40 d before maximum.

Table 1 Two characteristic times of four galactic novae: t_L is the time after maximum light at which the spectrum begins to be dominated by emission lines. T is the time before maximum light of the ejection of the main shell.

Nova	t_L (d)	T (d)
CP Lac	15	19
DI Lac	55	20
Nova Cyg 1975	35	35
Nova Serpentis 1970	95	40

Let $\rho = E_l/E_c$, where E_l and E_c are the energies emitted in the lines and in the continuum. For most novae there is a characteristic time t_L such that for $t > t_L$, $\rho > 1$. From data given in the literature it is very difficult to deduce the value of t_L for novae observed in the past. In four novae, however, whose spectra were recorded at the Wise Observatory in the past 2 yr (Novae Sag 1974, Per 1974, Scuti 1975 and Cyg 1975) it was found to vary considerably. Inspection by eye of spectrograms of Nova Cyg 1975 leads to an estimate of $t_L \sim 30$ d, after maximum brightness.

By the definition of t_L , the light curve of a nova at $t > t_L$ describes mainly the evolution of the intensity of the emission lines of the nova shell. If the permitted emission lines of novae, in particular those of hydrogen, are produced by ionisation and recombination, the time scale for intensity variations is the recombination time $\tau \approx (N_e \alpha)^{-1}$. Here N_e is the electron density and α is the recombination coefficient on all levels of hydrogen. At an electron temperature $T_e = 10^4$ K, $\alpha \approx 4 \times 10^{-13}$ cm³ s⁻¹ (ref. 2) and for $N_e < 3 \times 10^7$ cm⁻³ $\tau > 1$ d. An envelope with a density of 3×10^{14} cm⁻³ ejected at a velocity of 500 km s⁻¹ will reach this density in ~ 50 d. Many galactic novae have a 'transition' period in their light curve, during which rapid oscillations with a time scale of a few hours occur¹. If these oscillations are in the photospheric radiation they must be therefore at times $t < t_L$. There are on the other hand some novae whose light curves are smooth, showing no significant oscillations. It is suggestive to conjecture that in these novae t_L is small. Nova Cyg 1975 is a case in point as the small value of $t_L \sim 30$ d and the light curve in Fig. 1 indicate.

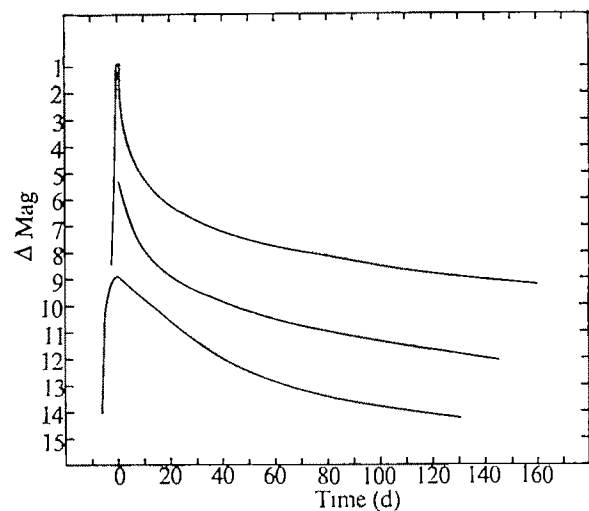


Fig. 1 Smoothed light curves of three novae. The curves of CP Lac (middle) and DI Lac (bottom) are from ref. 1. The curve of Nova Cyg 1975 (top) was constructed by Dr I. Kupo from data published in IAU circulars in 1975 and 1976. The magnitude units are on a relative scale.

The intensity of a recombination line is given by

$$I = \alpha_{eff} V N_e^2$$

where α_{eff} is the effective recombination coefficient for the emission of the line and V is the emitting volume². The intensity of a forbidden line at a given ionisation and temperature is also proportional to $V N_e^2$. For an expanding ionised hydrogen shell of a given mass, $N_e V = M$ is the mass of the shell in atomic units. Thus

$$I = \alpha_{eff} M N_e$$

For a thin expanding shell of invariant thickness

$$N_e(t) = N_e(0) [r_0 / (r_0 + vt)]^2$$

where $N_e(0)$ and r_0 are the density and the radius of the shell at $t = 0$ and v is the expansion velocity. Therefore

$$\frac{I(t_1)}{I(t)} = \left[\frac{r_0 + vt}{r_0 + vt_1} \right]^2$$

where t_1 is some reference time.

Let the zero of time be the moment of maximum light in the photometric V band, and let T be the time difference between the ejection of the main shell and maximum light. We assume that the acceleration time of the shell is $\ll T$. The ejection time is thus defined more strictly as the time at which most of the main shell acquires its final expansion velocity v . If the assumption is not

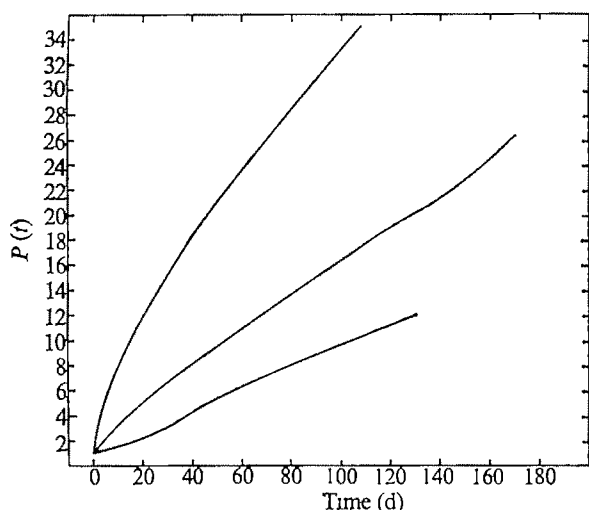


Fig. 2 The observed function $P(t) = [F(0)/F(t)]^{1/2}$ against t for three novae (in same order as in Fig. 1); $F(t)$ is proportional to the observed flux at the time t .

valid, T will be a lower limit to the time difference between ejection and maximum light. We therefore have

$$r_0 \simeq vT$$

At $t > t_L$ the flux F of the radiation of the nova in the V band is mainly the flux in the emission lines of the shell therefore for $t \geq t_1 \geq t_L$

$$\left[\frac{F(t_1)}{F(t)} \right]^{1/2} = \frac{1+t/T}{1+t_1/T} \quad (1)$$

For all $t > 0$ we define

$$P(t) = [F(0)/F(t)]^{1/2} \quad (2)$$

At $t \geq t_1 \geq t_L$ equations (1) and (2) give

$$P(t) = \alpha \times \beta$$

where

$$\alpha = \frac{[F(0)/F(t_1)]^{1/2}}{T+t_1}; \quad \beta = \frac{[F(0)/F(t_1)]^{1/2}}{1+t_1/T} \quad (3)$$

A plot of $P(t)$ against t should become a straight line at $t > t_L$. The parameters of the line give the time of the ejection before maximum light

$$T = \beta/\alpha$$

Figure 2 is a plot of $P(t)$ against t for the novae CP Lac, DI Lac and Nova Cyg 1975. These novae have no transition periods in their light curves, as shown in Fig. 1.

The curves in Fig. 2 do have linear sections and the parameters derived from them are summarised in Table 1. The parameters for Nova Serpentis 1970 are obtained from a similar analysis of the light curve of this nova, given by Hutchings³. The value of T for this nova is much less certain because of the small extension of the light curve beyond t_L .

The reality of the theory put forward here and of the results obtained from it is somewhat confirmed by the shape of the curves in Fig. 2 which follows the expected one. In particular it justifies empirically the assumption of invariant thickness of the expanding shell. An homologous expansion of a thin shell, for example, requires a change in the definition of the function $P(t)$ in expression (2) to $[F(t)/F(t)]^{1/2}$. With such a definition, the fit

of $P(t)$ to a linear expression in t is worse than the one obtained here. This is indicated by a smaller correlation coefficient for the fit for the four novae and by the systematic deviation of the distribution of the points from a straight line. Physically this assumption is justified because the velocity dispersion within gaseous shells of novae is probably much smaller than the expansion velocity. At times when the increase in the thickness of the shell is still small relative to the initial thickness, the thickness of the shell is to a first approximation invariant. An additional test can be performed by deriving the value of T directly from the value of α or from the value of β . Using expressions (3) we indeed obtain consistent values of T from both expressions. This is not a trivial result as α and β are two independent observational parameters.

An obvious improvement of the method suggested here is the recording of the intensity of a specific recombination line, for example H_β , with narrow band photometry, throughout the nova episode. Data points on such a light curve should be significant for this analysis even at $t < t_L$. They are also unaffected by the radiation in forbidden lines which is much more temperature dependent.

I am grateful to Dr I. Kupo for providing me with the light curve of Nova Cyg 1975.

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Radioactivity in the galactic plane

We report here what may be the first detection by a nuclear γ -ray transition of a large concentration of interstellar radioactivity. Such a concentration of radioactivity would be expected if heavy nuclei are synthesised explosively in supernovae. We do not have sufficiently conclusive counting statistics to make an incontestable claim, but the phenomenon we have observed in the background of our measurements is tantalisingly near to theoretical expectations.

The observation was made during balloon-altitude measurements of γ -ray energy spectra in the 0.02–12.27 MeV energy band from galactic and extragalactic sources. These measurements were conducted from Rio Cuarto, Argentina, during April 1–2, 1974. The measurements and the actively-collimated scintillation counter we used have been described elsewhere^{1,2}. In each measurement, a given source was observed for many time segments (each averaging 10 min) that we alternated with equal duration, equal zenith-angle measurements of the background. A galactic co-ordinates plot of the relevant background pointing directions is shown in Fig. 1 of ref. 1; the background series starts with segment³ 1 and ends with background segment 22. The tenth, eleventh and twelfth of these sequential background observations (hereafter labelled B10, B11, and B12) were made in directions towards the plane of the Galaxy—B10 has the lowest average galactic latitude—while the other backgrounds were measured at higher galactic latitudes. B10, B11 and B12 displayed enhanced counting rates relative to the other background segments, and an effort was made to ascertain the cause of the enhancement. This enhancement was most significant statistically at energies < 0.2 MeV, and most significant of all for B10. An energy spectrum was computed for B10 by treating it as resulting from a 'source' and subtracting from B10 a background (B8) measured ~ 30 min earlier at a higher galactic latitude. The 'B10 energy spectrum' is the product of this difference

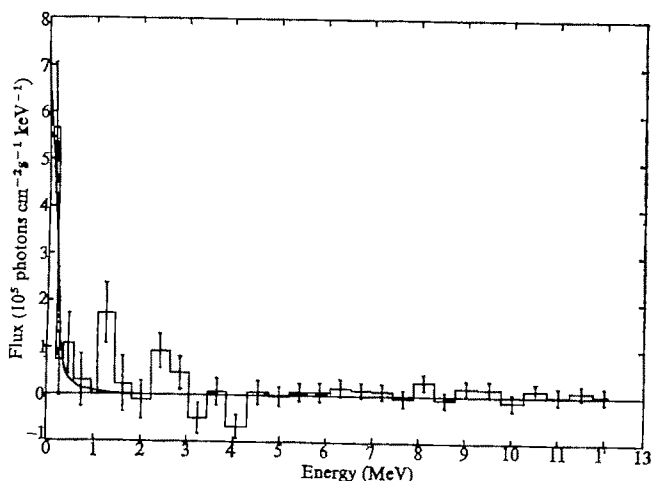


Fig. 1 B10 energy spectrum for April 2, 1974. Data shown are energy-dependent summations of individual pulse-height channels, the F.W.H.M. energy resolution at energies near 1 MeV is 5 individual channels, for example. Bins in the figure are 4.0 F.W.H.M. in energy < 1.0 MeV and 2.0 F.W.H.M. > 1.0 MeV. The best-fit power law (see text) is also shown. All error bars shown are $\pm 1\sigma$ and are purely statistical in character.

multiplied by the appropriate correction factors (Waraven *et al.*²) for atmospheric absorption and instrumental efficiency. Figure 1 shows the B10 energy spectrum with individual pulse-height channels summed (for clarity) as indicated in the caption.

When a power-law fit was attempted to all of the channels in the B10 energy spectrum, a reduced χ^2 value of 0.894 was obtained, for 64 d.f. This fit is $(9.5 \pm 2.6) \times 10^{-3} E^{-1.4 \pm 0.1}$ photons $\text{cm}^{-2} \text{s}^{-1} \text{keV}^{-1}$, where the photon energy, E , is expressed in keV. This off-the-galactic-centre continuum has a much harder spectral index than the index of 2.78 found¹ for the galactic centre region continuum. The present continuum spectrum equals, within statistics, a prolongation of the galactic-plane spectrum measured at higher energies on SAS-2 (ref. 4), allowing for our 0.04-sr acceptance angle.

Our curiosity was aroused by the spectral feature that seems to be present (Fig. 1) in the B10 spectrum in a 1.0 F.W.H.M. interval centred near an energy of 1.16 MeV; statistically, it is the only large deviation from the power law. (The spectral lines from excited, stable nuclei that we detected¹ from the central longitudes of the Galaxy are too faint to have been detectable in this brief observation.) There is a flux of 4.2×10^{-3} photons $\text{cm}^{-2} \text{s}^{-1}$ in this interval, which extends from 1.094 to 1.274 MeV. This flux is 2.6σ above that expected from the power law. A hypothesis of a Gaussian superimposed on the B10 power law was fitted to the individual channel data, and a flux amplitude of $(3.4 \pm 1.5) \times 10^{-3}$ photons $\text{cm}^{-2} \text{s}^{-1}$ was obtained, with the hypothetical line at an energy of (1.15 ± 0.07) MeV. This yielded a reduced χ^2 value of 1.195, for 12 degrees of freedom.

Instead of B8 alone, all backgrounds except B10 were combined into a weighted mean, and the weighted mean was then subtracted from B10 to form an energy spectrum. This procedure may not have very great physical significance, because the backgrounds were measured at various zenith angles and at different times. Because the weighted-mean counting rate is slightly higher than that of B8 in this energy interval, this weighted-mean spectral line flux— $(1.9 \pm 1.2) \times 10^{-3}$ photons $\text{cm}^{-2} \text{s}^{-1}$ —is somewhat lower than the line flux given above. The feature clearly lies in a positive excess in B10, rather than a negative departure in either B8 or the weighted-mean backgrounds. Because the statistical confidence is so low, $< 3\sigma$, meaningful comments on possible broadening beyond the instrumental broadening cannot now be made.

B10 seems to have an anomalous energy spectrum. The midpoint of segment B10 occurred when the axis of the detector was pointed towards galactic longitude 345° and the galactic latitude $+5^\circ$. (The estimated error in both coordinates is $\pm 1^\circ$.) In the B10 sky region, the beam of the experiment projects on to the sky as a circle with a 6.5° radius. Because of the very limited number (~ 125) of 'source' counts, the existence of a nuclear γ -ray line at 1.15 MeV in B10 cannot here be conclusively established. If a line is indeed the physical cause of our spectral feature, then ^{44}Ca , which emits γ radiation at 1.156 MeV following the decay of radioactive ^{44}Sc , is a likely candidate. This nucleus arises from ^{44}Ti according to explosive models of supernova nucleosynthesis⁵, and was predicted by Clayton *et al.*⁶ to be the most likely galactic γ -ray spectral line to be discovered. Only supernova remnants less than a few years old should emit more intense γ -ray lines. The 1.6-MeV line flux inferred from our data equals the predicted flux⁶ for a supernova at a distance of ~ 3 kpc and an age of $\lesssim 100$ yr. These numbers do not seem unreasonable, considering that the ^{44}Ti yield 'per average supernova' need not characterise the actual yield of an unknown event in the Galaxy.

In any background runs, 2.6σ departures from a fitted continuum will occur, merely from random fluctuations. It is at least a remarkable double coincidence, however, that just when the theoretically-predicted spectral anomaly occurred just when the background measurements crossed the galactic plane. We do not claim that positive evidence for nucleosynthesis in explosive galactic events has been detected, but are reporting these results in the hope that γ -ray astronomers will be stimulated to search the B10 portion of the Galaxy for the 1.16-MeV line with more sensitive equipment. If it does arise from ^{44}Ti , the associated ^{44}Sc -decay flux will be almost constant over our lifetimes.

If ^{44}Sc is responsible for the emission near 1.16 MeV, then a flux of comparable intensity is expected near 0.511 MeV, from positron annihilation. The B10 flux measured in the 0.44–0.52 MeV interval (which includes the positronium-decay spectrum⁷), is 5.1×10^{-4} photons $\text{cm}^{-2} \text{s}^{-1}$, which is within 1σ of the contribution from the power law in this interval. Evidently the available data are consistent with the presence of radioactive ^{44}Sc , but are insufficient to establish the existence of this species within the field of view.

Optical and radio data are inconclusive regarding the model of a supernova remnant as the source of the spectral anomaly. An optical supernova remnant has been identified by Van den Bergh *et al.*⁸ at galactic coordinates of 352.05° and $+0.13^\circ$, where there is an indication of faint filamentary structure together with an extended H II region. At 408 MHz, the remnant has an angular diameter of $30'$, which implies, for a 3-kpc distance, that the age is > 50 yr. This makes the remnant probably too old to be responsible for the observed anomaly. A much smaller source of 408-MHz radiation has been identified⁹, at galactic coordinates 345.34° and $+1.43^\circ$. Although no supernova remnant has been identified optically at these latter galactic coordinates, the source can be considered a more likely candidate for the cause of the B10 anomaly, since its absolute minimum age is only ~ 10 yr.

Further observations of this region of the Galaxy should be made using more sensitive γ -ray spectrometers; they may confirm an important step forward in understanding the origins of elements.

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The role of Lyman- α photons in the interstellar medium

LYMAN- α 1,216 Å photons may be produced in a variety of astrophysical situations. These photons are lost through two-photon emission on interacting with hydrogen gas, and through absorption by interstellar grains, but velocity differences between the regions of production and destruction of these photons renders their destruction by the two-photon emission process relatively inefficient¹. Grain absorption is therefore the primary loss mechanism, but since it occurs over path lengths $\gtrsim 50$ pc, there are significant interstellar Ly- α fluxes. An early theoretical estimate¹ of the interstellar flux of diffuse Ly- α photons yielded a value $\sim 6.4 \times 10^{-4}$ erg cm⁻² s⁻¹ sr⁻¹. This estimate agrees well with the first Mariner-5 and Mariner-6 observations of this flux by Barth^{2,3}, the latter giving a value of $\sim 4.9 \times 10^{-4}$ erg cm⁻² s⁻¹ sr⁻¹. Later authors^{4,5} have queried the precise fraction of this observed radiation which is genuinely extra-solar, although a significant interstellar component of Ly- α flux is generally conceded. A value in the range $1\text{--}4 \times 10^{-4}$ erg cm⁻² s⁻¹ sr⁻¹ would appear consistent with the available data. We note that the energy flux in Ly- α at the lower limit of this range is already ~ 5 times the average continuum of diffuse radiation⁶ in a 100-Å band pass centred on $\lambda = 1,425$ Å. It is also ~ 10 times greater than theoretical estimates of the continuum flux in the same waveband^{7,8}. Such a large Ly- α flux is difficult to understand if it arises from the Lyman continuum of O-B stars. Münch's original estimate¹ was based on the integrated effect of stellar chromospheres, the Ly- α photons being produced by non-thermal processes. Other likely sources of non-thermal Ly- α radiation include production in interactions of stellar winds with the interstellar medium, supernovae remnants and O VI regions such as indicated by recent Copernicus observations^{9,10}. Lyman- α photons are also expected to be formed in intercloud regions where the production of such photons provides the main cooling process. Here we tentatively adopt a Ly- α flux in the range $\sim (1\text{--}4.0) \times 10^{-4}$ erg cm⁻² s⁻¹ sr⁻¹ as being appropriate for the general interstellar medium. It is of interest that the energy density associated with the upper end of this flux range is $\sim 1.68 \times 10^{-13}$ erg cm⁻³, ~ 0.25 of the total energy density of starlight in the Galaxy. Furthermore, we note that this energy density is of the same order of magnitude as that appropriate for the galactic magnetic field, the turbulent motion of interstellar gas and the cosmic microwave background. We show here that this high Ly- α flux is of basic importance for the physics of the interstellar medium.

The electrostatic charge on grains is an important parameter which influences grain-plasma interactions, and also, probably, the heating rate of the interstellar medium. The mean equilibrium charge on grains for several likely grain materials is expected to be determined by a balance between photoejections caused by ultraviolet photons and the capture of ambient electrons^{11–13}. A mean grain potential of several tenths of a volt has been calculated¹² using the observed flux of continuum diffuse radiation in the waveband 10–13.6 eV, and for a range of interstellar cloud conditions. The mean equilibrium potential

(considering only a balance between photoejections and recombinations) is given by

$$\bar{\Phi} = kT \left\{ \left[\int_{E_i}^{\infty} F_{\gamma} Q_{abs} dE_{\gamma} / n_e v_e S \right] - 1 \right\} \quad (1)$$

where $F dE_{\gamma}$ is the radiation flux in the photon energy range E_{γ} , $E_{\gamma} + dE_{\gamma}$. Q_{abs} is the grain absorption efficiency, γ is the photoelectric yield, n_e , v_e , T are the electron density, velocity and temperature, S is a sticking coefficient and E_i is the photoelectric threshold. The value of E_i is typically ~ 6 eV for metals (including graphite) and ~ 9 eV for insulators such as silicates. In both cases these threshold energies are less than the Ly- α photon energy, $E_{\alpha} \simeq 10.2$ eV. The photoelectric yield for small particles is a factor ~ 3 higher than that for bulk material¹⁴. Taking this factor into account we adopt representative values of 0.1 for silicates and 3×10^{-3} for graphite using data of Willis *et al.*¹¹, for photon energies close to 10.2 eV. For the range of interstellar diffuse Ly- α flux adopted here the first term within brackets in equation (1) is changed by a factor of $\sim 0.4\text{--}4.3$ from its value assuming only a continuum ultraviolet flux⁶ 2×10^{-7} erg cm⁻² s⁻¹ sr⁻¹ Å⁻¹. We then obtain a mean steady-state grain potential

$$\begin{aligned} \bar{\Phi} &= \left\{ \frac{0.1 \xi \gamma}{n_e} - 0.0086 \right\} \quad V, T = 100 \text{ K} \\ &= \left\{ \frac{\xi \gamma}{n_e} - 0.86 \right\} \quad V, T = 10^4 \text{ K} \end{aligned} \quad (2)$$

where ξ is in the range 1.7–6.8 adopting $S = 1$, with the proviso that $\bar{\Phi}$ cannot exceed Φ_{max} given by

$$e\Phi_{max} \simeq E_{\alpha} - E_i$$

The values of Φ_{max} for silicates and graphite are respectively ~ 1.2 and 4.0 V for the assumed values of E_i . Setting $n_e = 10^{-3}$ cm⁻³ we obtain the following values of $\bar{\Phi}$: for $T = 10^2$ K, the potential for silicates is ~ 1.2 V and for graphite 0.04–0.20 V; while for $T = 10^4$ K, for silicates it is ~ 1.2 V, and for graphite $-0.35\text{--}1.12$ V. These calculations involved only an equilibrium between photoejection and recapture of electrons by grains. A more rigorous determination of $\bar{\Phi}$ should take account also of the capture rate of positive ions. Such a procedure does not change our estimates of $\bar{\Phi}$ for silicates. For graphite, however, slight departures occur, but these are of negligible relevance in the ensuing discussion.

For silicate grains with a relatively high photoemission yield the mean potential saturates at ~ 1.2 V in both H I clouds and intercloud regions. The close equality of this potential to $(E_{\alpha} - E_i)/e$ would preclude a significant heat source from ejected photoelectrons if all grains acquired the same potential. A stochastic distribution about a mean grain potential is, however, expected to occur¹⁴, and the r.m.s. energy of emitted photoelectrons has a non-zero value which we estimate as $E_e \simeq 0.5$ eV. The heating rate Γ_e from photoelectron emission from silicate grains by Ly- α photons is

$$\Gamma_e = \frac{\sigma n_s}{n_H} Q_{abs} \gamma E_e F_{\alpha} n_H \text{ erg cm}^{-3} \text{ s}^{-1} \quad (3)$$

where σ is the geometrical cross section, Q_{abs} is the absorption efficiency of grains, n_s is the grain density, n_H is the hydrogen density, γ is the photoelectron emission efficiency, E_e is the

mean energy of emitted photoelectrons and F_α is the flux of Ly- α photons. For $y = 0.1$, $E_c = 0.5$ eV we obtain

$$\Gamma_a = (0.38-1.5) \times 10^{-26} \left\{ \frac{\sigma n_g Q_{ab}}{n_H (6 \times 10^{-22} \text{ cm}^2)} \right\} n_H \text{ erg cm}^{-2} \text{ s}^{-1} \quad (4)$$

for F_α in the adopted range.

With $\sigma n_g Q_{ab}/n_H \simeq 6 \times 10^{-22} \text{ cm}^2$ (ref. 15) we get from equation (4) a heat input rate which is significantly higher than the corresponding rate calculated for photoemission by ultraviolet continuum photons. For graphite grains with $|\Phi| \lesssim 1.0$ V (in both cloud and intercloud regions, $y = 3 \times 10^{-3}$ and $E_c \sim 4$ eV) we find

$$\Gamma_a = (0.1-0.36) \times 10^{-26} \left\{ \frac{\sigma n_g Q_{ab}}{n_H (6 \times 10^{-22} \text{ cm}^2)} \right\} n_H \text{ erg cm}^{-2} \text{ s}^{-1} \quad (5)$$

Again with $\sigma n_g Q_{ab}/n_H \simeq 6 \times 10^{-22} \text{ cm}^2$ we obtain a heating rate which is consistent with available data on gas temperatures and cooling rates in both clouds and intercloud regions. The observed high flux of Ly- α photons could thus provide a major source of heating for the interstellar medium through their photoelectric effect on grains.

The line-centre photons of Ly- α cannot ionise hydrogen atoms, which have an ionisation threshold wavelength of 912 Å. Because of the natural width of the emitted and scattered line photons, there would be photons with energies > 13.6 eV in the line wing which could contribute to the ionisation rate of the interstellar gas. We can show, however, that the contribution of this process is not very significant compared with other mechanisms which have been proposed unless the Ly- α flux is equal to or higher than the larger value adopted in the preceding discussion.

We conclude that the recently observed large flux of diffuse galactic Ly- α photons would have an important effect in controlling physical processes in the interstellar medium. In particular the electric charge on grains, particularly silicate grains, will be primarily determined by photoejections caused by Ly- α photons. The heating of the interstellar medium will also mainly be caused by this process. If external galaxies emit an equally high fraction of their luminosity in Ly- α radiation as is indicated in our own galaxy, their effect on the intergalactic medium as well as their possible cosmological role will need to be considered. A more precise determination of the Galactic Ly- α flux is clearly a matter of paramount astrophysical importance.

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Primitive grain clumps and organic compounds in carbonaceous chondrites

We show here that the physical conditions in prestellar molecular clouds favour the condensation of complex organic polymers, including amino acids, within a matrix of smaller refractory particles. Such composite grain clumps with dimensions exceeding 1 μm could be expelled along with gaseous material in protostellar cocoons, causing the widespread dispersal of biological activity in the Galaxy. We argue that grain clumps of the type considered here may be identified with μm -sized inclusions in carbonaceous chondrites.

Carbonaceous chondrites, with a carbon content of several per cent mainly in the form of aromatic polymers, and including amino acids in trace quantities, are generally believed to be among the most primitive solid bodies in the Solar System. The compaction of mineral particles with a substantial admixture of trapped volatiles must have occurred at temperatures in the range 350–500 K, with no subsequent reheating above ~ 500 K (refs 1 and 2).

Several striking isotopic anomalies have been discovered in mineral separates from carbonaceous chondrites^{3–7}. Such anomalies have tentatively been attributed to inclusions of interstellar grains which condensed in novae or supernovae explosions^{3,6,8,9}. This explanation is consistent with the occurrence of heavily irradiated μm -sized mineral separates rich in ²²Ne in the Orgueil meteorite¹⁰. The presence of μm -sized inclusions, each comprised of closely packed aggregates of grains of 100 Å (ref. 11), is also suggestive of interstellar grain clumps within carbonaceous chondrites.

An understanding of the origin of carbonaceous chondrites may have an important bearing on the early history of the solar nebula, and in particular on theories of planetary formation. The efficient adhesion of relatively cold refractory grains (for example, graphite or silicate particles) in low velocity grain-grain collisions could occur if these grains possessed mantles composed of organic polymers which are adhesive at temperatures ~ 300 K. Such organic polymers have been tentatively identified by their infrared spectral features in cometary as well as interstellar dust^{12,13}.

We argue here that interstellar molecular clouds which are the most probable sites for the condensation of polymeric mantles around grains are also likely to provide suitable venues for the formation of composite grain aggregates, by the adhesion of such coated grains in grain-grain encounters. Such grain clumps of sizes $\sim 1 \mu\text{m}$ pre-existing in the solar nebula could have served as aggregation centres for the growth of carbonaceous chondrites, perhaps representing the earliest stage of planet formation.

Large molecular clouds with masses in the range $\sim 10^4$ – $10^6 M_\odot$ are widespread in the galactic disk. Such clouds, typified by W3, OMC-2, NGC2024, Sg B2, are generally believed to be progenitors of OB associations. In a typical extended cloud of diameter ~ 10 pc, observations of molecular CO at millimetre wavelengths leads to an estimate $n_{\text{H}_2} \simeq 3 \times 10^3 \text{ cm}^{-3}$ for the smeared out hydrogen density¹⁴. More complex molecules, including HCN, H_2CO , tend to be more localised in their spatial distribution, generally associated with infrared knots, OH masers and presumably protostellar clouds. Molecular densities in such clouds are difficult to estimate. The requirement for collisional excitation of optically thin lines of H_2CO , HCN by neutral particles gives a lower limit $n_{\text{H}_2} > 10^5$ (ref. 14), but densities $\sim 10^6 \text{ cm}^{-3}$ or higher are most probably appropriate to protostellar clouds.

One may also argue that molecular clouds are not in a state of free-fall collapse¹⁵. Condensation may be slowed down by several processes, including effects of magnetic pressure, rotation and turbulence. We assume here that typical collapse times for an entire cloud, as well as for fragments within it, are of the general order of 10^4 yr. Such a condensation time, together

with the estimated total mass of protostellar clouds, gives a rate of star formation which is consistent with observations.

A molecular cloud fragment collapsing towards a protostellar situation will contain a mass fraction of $\sim 10^{-3}$ of refractory grains such as graphite, silicate and iron particles of mean radius $a_1 = 2 \times 10^{-6}$ cm. The first stages of collapse will be accompanied by accretion of organic molecules on to these grains. Since a significant mass fraction of C and O is initially in solid grains, the maximum extent of mantle growth is not likely to exceed 50% of the original radius. This gas phase accretion would proceed to effective completion on a time scale which is short compared with the estimated collapse time of $\sim 10^4$ yr. The grain radius may now be assumed to be 3×10^{-6} cm (50% increase) in accord with our earlier remarks. The precise composition of molecular mantles is uncertain, but a hybrid mixture of organic polymers is likely to ensue.

Refractory grains with such tar-like polymeric coatings tend to stick to one another in low velocity grain-grain collisions at temperature $T \approx 300$ K. Suppose $n_H (= 2n_{H_2})$ is the total hydrogen density and n_g is the grain density at this stage of protostellar collapse. Assuming an initial grain mass fraction of $\sim 1\%$, we have (for any reasonable grain specific gravity)

$$\frac{n_g}{n_H} \approx 3 \times 10^{-10} \quad (1)$$

The rate of growth of a grain clump of radius r by this process is given by

$$\begin{aligned} \frac{dr}{dt} &= \frac{\alpha n_g}{s} \left[\frac{kT \left(\frac{4}{3} \pi a_1^3 s \right)}{2\pi} \right]^{\frac{1}{2}} \\ &= \alpha n_g \left[\frac{2kTa_1^3}{3s} \right]^{\frac{1}{2}} \end{aligned} \quad (2)$$

where α is the sticking probability, s is the mean specific gravity of the grain clump material, $a_1 (= 3 \times 10^{-6}$ cm) is the radius of polymer coated grains, n_g is the number density of grains, and T is the kinetic temperature. We assume in equation (2) equipartition of energy between grains and gas and a Maxwellian distribution of grain velocities. Sticking of grains occurs by collisions during their Brownian motion with relative speeds of ~ 10 cm s $^{-1}$. With $\alpha \approx 1$, $T \approx 300$ K, $s = 1$, $a_1 = 3 \times 10^{-6}$ cm and using equation (1) we obtain

$$\frac{dr}{dt} = 8.2 \times 10^{-18} n_H \text{ cm yr}^{-1} \quad (3)$$

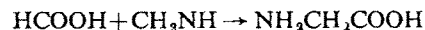
In the available time, $\sim 10^4$ yr, we obtain clump diameters $2r \sim 1 \mu\text{m}$ for a typical value of the molecular density $n_{H_2} \approx 3 \times 10^6$ cm $^{-3}$. Larger clumps could arise from higher density regions

The ultimate dispersal of a protostellar cocoon, including large grain clumps, may have a role in the removal of angular momentum from a central protostellar condensation, thus permitting further contraction and evolution on to the main sequence. A large fraction of composite grain clumps in such cocoons could probably survive the 'switching on' of the stars in an OB association, and they may be carried along with systematic gas flows into the general interstellar medium. Such grain clumps could indeed constitute an appreciable fraction by mass of all interstellar dust.

Large grain clumps of the type discussed here in a protoplanetary disk could also serve as accretion sites for smaller grains which condense within the disk, the process leading to the formation of planetesimals in the first instance, and eventually to planets. With a minor degree of metamorphism, such objects at

an intermediate stage of aggregation would seem to resemble the carbonaceous chondrites.

It is tempting to speculate that amino acids which have been discovered in carbonaceous chondrites¹⁵⁻¹⁷ had their origin in presolar grain clumps of the type considered here. The formation of simple amino acids (for example, glycine) is expected to take place in dense interstellar molecular clouds which may well be the cradle of life. The possible precursors of glycine, namely formic acid (HCOOH) and methanimine (CH $_2$ NH), have already been observed in dense molecular clouds, and the reaction



leading to the production of glycine is known to be exothermic. It may be relevant that glycine is the most abundant of the amino acids detected in chondrites¹⁷. Amino acids of this type, and of greater complexity may be trapped in the tarry polymeric component of our grain clumps, and be dispersed throughout interstellar space, being securely protected from destruction by ultraviolet photons by the matrix of smaller refractory grains in which they are embedded.

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Comparative ionospheric and plasmaspheric electron contents from three world regions

THE radio beacon experiment¹⁻³ aboard the geostationary Applied Technology Satellite ATS-6 enables the determination of total electron content (TEC) to be made by two independent methods. The first—the Faraday polarisation rotation technique—has been widely used in obtaining TEC data. Faraday rotation is dependent on the Earth's magnetic field, and, since its magnitude is heavily weighted near the Earth, it is considered to provide integrated electron content values for altitudes below $\sim 1,500$ km. The second—the dispersive-group-delay technique, in which the phase of the modulation envelope between a carrier and its sideband is compared at two frequencies—is independent of the Earth's magnetic field and thus yields the integrated electron content between the observer and the satellite signal source. The Faraday content, N_F , and the dispersive-group-delay content, N_T , therefore, yield the TEC up to $\sim 1,500$ km and geostationary altitudes, respectively. The difference between N_T and N_F yields the content above $\sim 1,500$ km, which is referred to as the plasmaspheric content, N_P .

The ATS-6 satellite was launched into a geostationary orbit in May 1974. During phase I of its operation, when

the satellite was located at 94°W, the US Army Electronics Command operated observation stations at Fort Monmouth, New Jersey (40.18°N, 74.06°W); Richmond, Florida (25.60°N, 80.40°W); and Sao Paulo, Brazil (23.50°S, 46.50°W). In May 1975, the satellite was moved to 35°E for phase II of its operation. During this phase, the observation stations were moved to Haifa, Israel (32.87°N, 35.09°E) and Kiruna, Sweden (61.84°N, 20.41°E). In August 1976, the satellite was moved to its final parking position at ~134°W, with some of the observation stations being reactivated and others being established.

The Faraday observations were made with the 140-MHz satellite beacon emissions. The dispersive-group-delay obser-

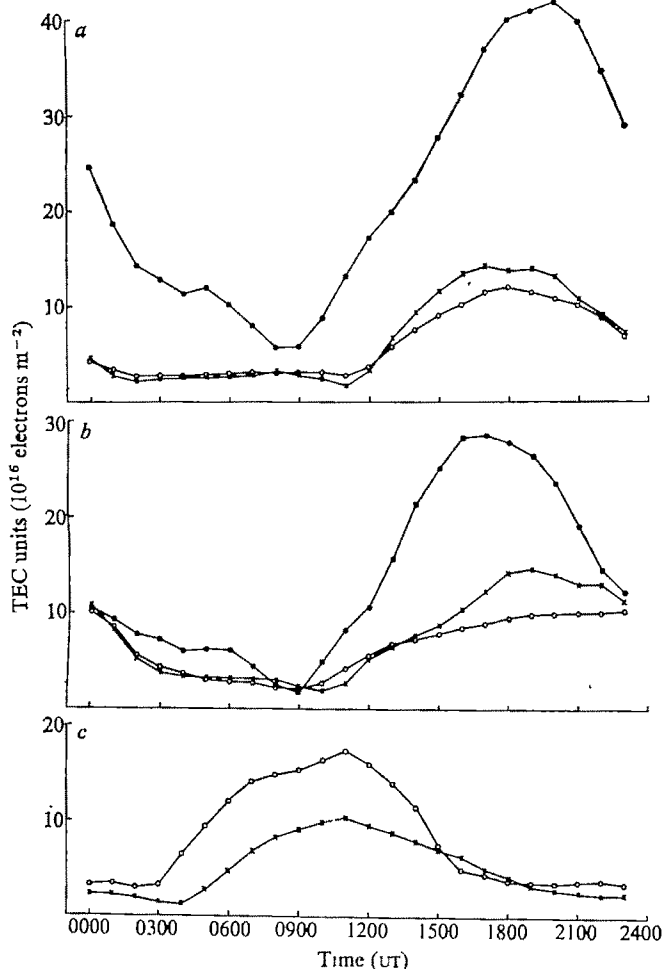


Fig. 1 *a*, Hourly mean monthly variation of N_F at Sao Paulo, (●) (LT = UT-3); Fort Monmouth, New Jersey (○) (LT = UT-5); and Richmond, Florida (×) (LT = UT-5), during February 1975 *b*, Same as *a*, but for May 1975 *c*, Hourly mean monthly variation of N_F at Kiruna, Sweden (×) (LT = UT+1); and Haifa, Israel (○) (LT = UT+2), October 1975.

variations were made with the 140 and 360-MHz beacons modulated with sideband displacement of $\Delta f = +1$ MHz.

Figure 1*a* and *b* depict a comparative mean monthly variation of TEC measured by the Faraday technique at Fort Monmouth, Richmond, and Sao Paulo for February 1975 and May 1975, respectively. The solar zenith angle dependence of TEC is shown in the relative values of TEC recorded at the two mid-latitude stations in the Northern Hemisphere. The comparatively higher TEC values recorded at Sao Paulo, even if seasonal effects are ignored (compare Fig. 1*a* and *b*), were due to the passage of the ray path from satellite to observer through the Equatorial Anomaly region. At equatorial regions, ionospheric plasma which moves upwards from an imposed east-west electric field diffuses downwards again, but obliquely, along the geomag-

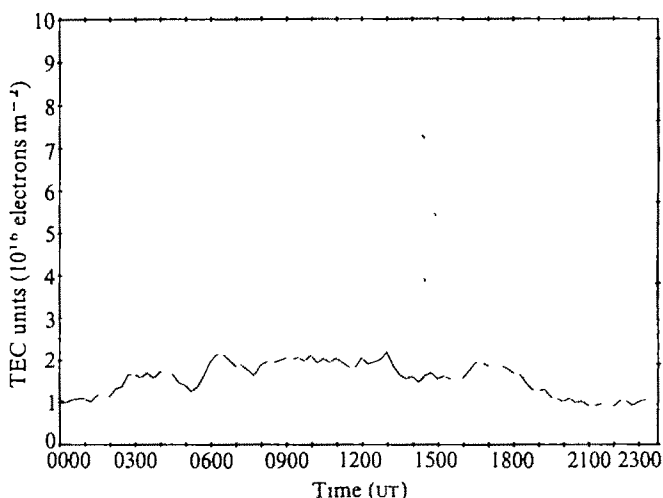


Fig. 2 Mean monthly variation of N_F at Kiruna, Sweden (at 15-min intervals), October 1975.

netic lines of force. Instead of returning to the source, the plasma arrives at two places—one to the south and one to the north of the equator. The electron content is then depleted at the magnetic equator, but is enhanced at the anomalous regions. The relatively higher TEC values for February as compared to May, confirm topside sounder results⁴ that the anomaly content crests are larger in the summer hemisphere. In addition, the ray path was in the vicinity of the South Atlantic Anomaly region where the magnetic field is abnormally weak and the loss of radiation belt particles is rapid. TEC enhancements are attributed to ionisation produced by the precipitating energetic trapped electrons that mirror low in the anomaly^{4,9}.

The comparative variation of TEC at a mid-latitude station and at a station near the auroral zone is shown in Fig. 1*c* for October 1975. The consistently higher TEC values at Haifa were due to the influence of the solar zenith angle on TEC. The passage of the ray path from Kiruna to the satellite through the density trough^{7,8} was also responsible for the observed depressed TEC values.

The mean monthly variation of the plasmaspheric electron content at Kiruna, Sweden, for October 1975 is shown in Fig. 2. The maximum content was during the day and the minimum at night, with average values between ~1.0 and ~2.0 TEC units.

Finally, the comparative variation of the plasmaspheric electron content, N_F , at Fort Monmouth and Sao Paulo during May 1975 is shown in Fig. 3. In general, the mean monthly values of N_F at both stations lay between ~1 and ~2 TEC units. The mean diurnal variation at Sao Paulo indicates that N_F had higher values during the day than during the night. The times of N_F enhancement were close to those at which the equatorial anomaly is formed and maintained⁴, and the time of N_F depression corresponded to

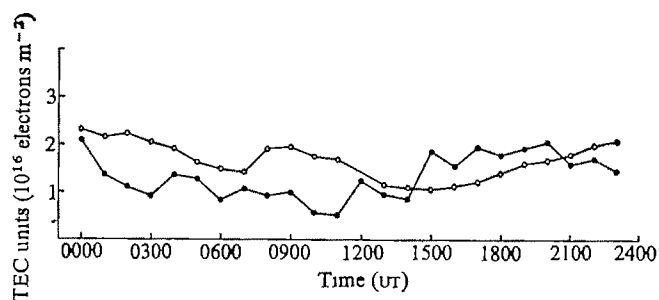


Fig. 3 Mean monthly variation of N_F at Fort Monmouth, New Jersey (○); and Sao Paulo, Brazil (●), May 1975.

disappearance of the anomaly. It is therefore possible, that during the formation of the anomaly and during its maintenance, the plasmaspheric and the ionospheric electron densities are increased.

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Meridional interactions between rainfall and surface pressure

We have been investigating temporal variation in rainfall over a part of the Southern Hemisphere^{1–4}. In view of the current concern regarding food production and future climatic change (for example, a shift in the westerlies over the Northern Hemisphere) this area of research is of some importance. The recent drought and resulting famine in the Sahel^{5–7} also shows that stresses in one hemisphere can be a burden on the food resources of the other. Until now, however, quantitative interactions between rainfall and the general circulation have not been given; here we show first, that there are structural relationships between annual rainfall over Southern Africa and the month to month variation in the mean latitude of the surface high pressure belt, L (ref. 8). The evidence adds credibility to findings related to the temporal variation in rainfall^{3,4}. Second, a means of forecasting annual rainfall totals is shown to be feasible. The method used in the analysis is neither geographically restricted, nor applicable to rainfall only.

A linear step-wise regression analysis was first carried out on 30 yr of rainfall data and the associated value of L (1942–71). In this way the independent variables, L , can be quickly assessed for their importance in predicting rainfall. The analysis was carried out on the total annual rainfall for the regional temporal series A as defined in refs 2 and 3. This time series represents the rainfall over an area whose middle is at $\sim 27^\circ\text{S}$.

We will give the months in the order they were taken into the regression. When the total rainfalls were regressed on the monthly L values, January and September were included. The fit of the regression is significant at the 2.5% level. To check for some sort of organisation in rainfall totals unaccounted for by the regression, a plot of the residuals was carried out. There is evidence of an oscillatory pattern in the residuals, the period being 2–3 yr. This may

be related to the quasi-biennial oscillation which is now a well accepted geophysical phenomenon⁹.

A regression of rainfall totals on L values during months in the preceding year resulted in the inclusion of June and March. The fit is significant at the 1% level. A plot of the residuals again indicates a wave-like pattern with period in the range 2–3 yr.

Mean latitudes, L , for one year, together with those for all months in the previous year, were then included in the regression, bringing the L -value for January of the year in question as well as June, March and February of the preceding year. The fit of this regression was significant at the 1% level. The predictors are not just the addition of the two sets from the previous regressions, which results from the use of the multiple correlation coefficient between rainfall and L (ref. 10).

As a forecasting equation this particular grouping of variables accounts for 52% of the total variance in annual rainfall. This is an encouraging result. A plot of the residuals against time indicated that a linear term was required in the regression. Such a term was added to the set of predictor variables and the analysis repeated, with the linear trend as the fifth term, or that of least importance. The

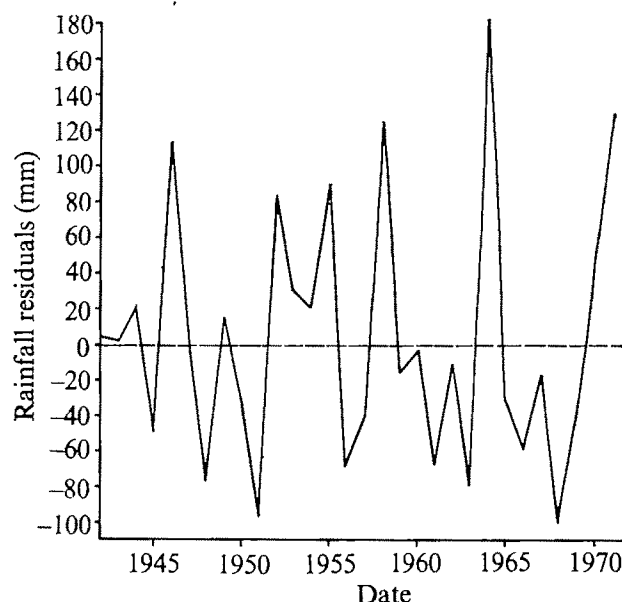


Fig. 1 Showing the rainfall residuals in a regression on the mean latitude of the surface high pressure belt for January in a current year, and June, March and February of the preceding year; the linear trend has been removed.

total variance now accounted for 58% of the whole. The residuals still exhibited an oscillatory pattern (Fig. 1) and they were therefore submitted to spectral analysis. Because of the short length of the series (30 terms) Burg's maximum entropy method was used¹¹. The spectrum shows excessive variance associated with periods of 5.88, 3.03 and 2.22 yr (Fig. 2).

A trigonometric function was fitted to the residuals, and resulted in the use of periods of 6.0 and 3.0 yr. The shortest period was considered too weak to include in the equation. The additional variance accounted for by this step was significant at the 1% level. In all, just over 84% of the total variance in rainfall has been accounted for, and the standard errors for forecasts is 6 cm, which is quite acceptable.

The final forecasting equation therefore contains as predictor variables:

(1) The mean latitude of the surface high pressure belt

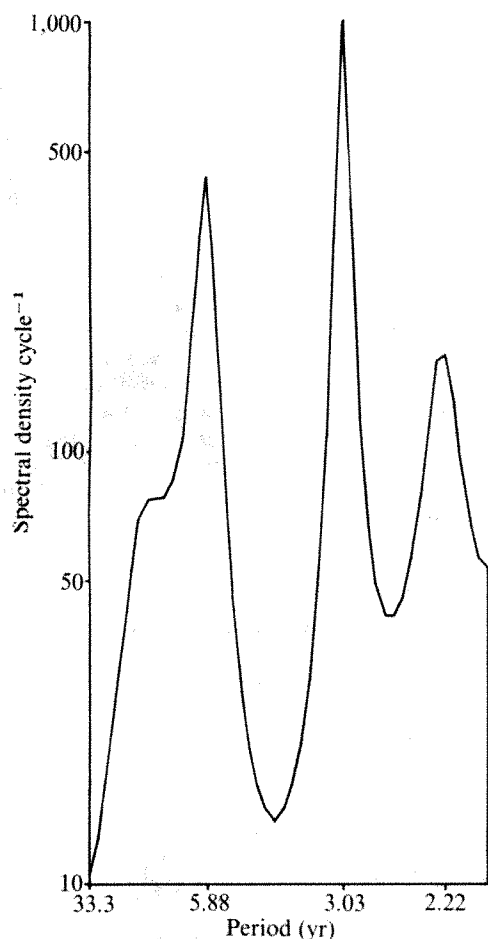


Fig. 2 Showing the spectrum of residuals when annual rainfall totals are regressed on the mean latitude of the surface high pressure belt for January in a current year, and June, March and February of the preceding year.

for January of a current year, with regression coefficient equal to -15.24 .

(2) As above, but for June, March and February of the preceding year, with regression coefficients -9.31 , 19.41 and 13.96 respectively.

(3) A linear term in time with regression coefficient 3.69 .

(4) A constant term equal to -422.82 .

(5) A trigonometric function having periods of 6.0 and 3.0 yr with cosine and sine coefficients 7.28 and -49.03 , and -47.82 and 39.04 respectively.

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"Upper Jurassic" sediments of South Africa

MARINE sedimentary rocks exposed near Knysna in the Cape Province of South Africa were assigned to the Upper Jurassic on the basis of a fragment of an ammonoid and seven previously undescribed species of ostracod¹. A more thorough examination of the microfossils in these rocks indicates that this assignment was erroneous and that the beds are of Early Cretaceous, probably Hauterivian, age². This age determination was based on identification of fifty species of palynomorph, five species of calcareous nannofossil, and twelve species of foraminifera. Both the palynomorphs and the foraminifera indicate a correlation with the upper part of the Sundays River Formation, a unit reliably dated as Valanginian to Hauterivian. The nannofossils indicate a Valanginian or Hauterivian age.

Ordinarily, a slight error in the age of a few small exposures in a remote portion of the globe would not be a matter of significance. These were the only marine sedimentary rocks of Jurassic age known from South Africa, however, and their age was considered to be important for timing the break-up of Gondwanaland³. In a very superficial search of the literature, we noted numerous citations of the Jurassic age (see refs 3-7). It was therefore thought desirable to bring to the attention of those concerned with the problems of Gondwanaland rifting that there are no marine sedimentary rocks of known Jurassic age in South Africa.

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Cyanophycean nature of stromatoporoids

STROMATOPOROIDS are calcareous fossils abundant in many Palaeozoic (mainly Silurian and Devonian) shallow-water carbonate sediments. Forms ascribed to stromatoporoids have also been reported from Mesozoic epicontinental and tethyan carbonate deposits¹. The nodular, tabular or cylindrical and, generally, vertically laminated calcitic skeletons of stromatoporoids (Fig. 1a), ranging in size from a few mm to >1.0 m, are internally formed of curved plates, short or long pillars and vertical walls. A characteristic feature of many stromatoporoids are stellate structures, a few mm to 6-7 cm across, termed astrorhizae. In spite of 150 yr of investigation the systematic position of stromatoporoids is still controversial. They have been variously interpreted as foraminifers, sponges and hydrozoans^{2,3} with discussion on the latter two groups bearing particularly on the interpretation of the skeletal morphogenesis^{4,5}. New discoveries⁶ of peculiar modern sponges with mixed calcareous and spicular siliceous skeletons (Sclerospongiae) reopened discussion on the relationship of this group to stromatoporoids, initiated at the beginning of the century by Kirkpatrick^{7,8}. Hartman and Goreau's suggestion⁹ has been taken up and supported by Stearn^{5,10} and Wendt¹¹. Stearn argued that stromatoporoids should be recognised as a separate subphylum of the Porifera. Wendt similarly regarded stromatoporoids as

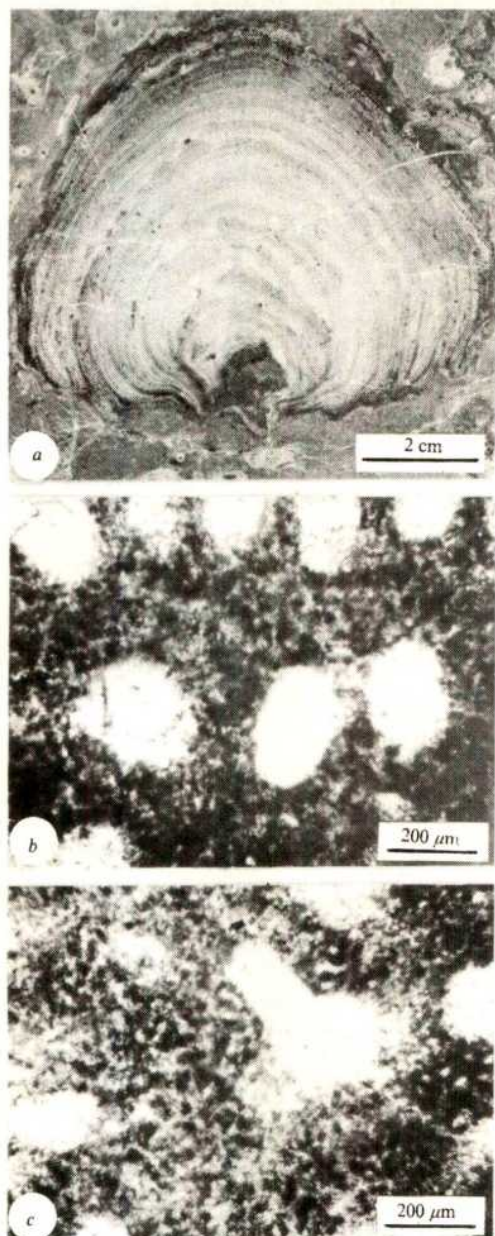


Fig. 1 *a*, Vertical axial section of the distinctly laminated skeleton of *Stromatopora undata* Riabinin from the Upper Devonian of central Poland; *b*, *c*, enlarged fragments of the same skeleton in vertical (*b*) and tangential (*c*) section showing granular microfabric representing permineralised aggregates of coccoidal cells, transmitted light.

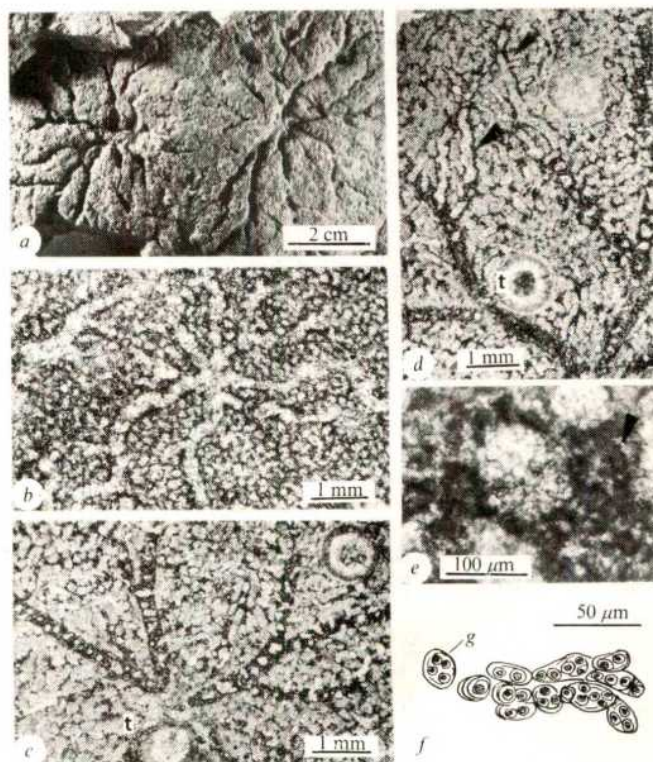
a conservative order of the Calcispongiae closely related to the Pharetronida. Spicules, however, are quite unknown in stromatoporoids and the discovery of fossil (Cretaceous) sclerosponges with preserved spicules^{12,13} shows that their absence cannot arise from diagenetic dissolution as Hartman and Goreau suggested. Interpretation of the astrophorizae is critical to any discussion of stromatoporoid affinities. As poriferans, the astrophorizae become traces left by the sponge exhalant canals, or as hydrozoans they are structures homologous to coenosarcial stolons (hydrohorizae). Kaźmierczak¹⁴ rejected both interpretations and argued that astrophorizae are traces of foreign organisms probably commensal or mutualistic with stromatoporoids. The new observations reported here modify but confirm this.

Discovery of unusually well preserved astrophorizae reveals the nature of stromatoporoids as permineralised colonies of coccoid cyanophytes homologous with modern and fossil

stromatolites. The astrophorizae occur in *Parallelopora* aff. *planulata* (Hall and Whitfield) obtained from the Upper Devonian (Frasnian) limestone of the borehole Niesiołowiec IG-1 (south-eastern Poland) at a depth of 1,330–1,336.4 m. In contrast with previously known astrophorizae, which appear either as a stellate system of furrows on the surface of the skeleton (Fig. 2*a*) or as radiating tubes with rare cross partitions filled by sparry calcite in thin section (Fig. 2*b*), the astrophorizae in *P.* aff. *planulata* are almost wholly filled by granular calcareous material (Fig. 2*c*). In transmitted light this is distinctly darker than the surrounding skeleton against which it sharply abuts. At many points, however, the skeleton passes into the dark astrophorizal infilling. The structural uniformity of the astrophorizal infilling and the surrounding skeleton is also stressed by the similar size and outline of the interskeletal spaces in both tissues. Where the dark granular material is absent the astrophorizal canals are filled by spar as is generally the case (Fig. 2*d*, arrowed). Under higher magnification the clotty infilling of the astrophorizae can be recognised as aggregations of spherical and subspherical bodies, slightly darker peripherally. Individual bodies have an average diameter of 10–40 μm and are distributed irregularly, rarely forming short pseudofilamentous rows (Fig. 2*e*, arrowed).

The globular bodies from the astrophorizal infillings and the granules of the surrounding skeleton are essentially similar, the main difference lying in the higher crystallinity of the

Fig. 2 *a*, Large astrophorizae on the surface of the Middle Devonian stromatoporoid *Trupetostroma* sp.; *b*, astrophorizae in tangential thin-section of Silurian *Plectostroma* sp. showing tubes filled by sparry calcite, transmitted light; *c*, *d*, tangential thin-sections through astrophorizal tubes in the Devonian *Parallelopora* aff. *planulata* (Hall and Whitfield) filled by dark granular material which is locally absent (arrowed), transmitted light. (Note also cross sections (t) of intergrown syringopoid tabulate corals); *e*, enlarged fragment of astrophorizal infilling showing subcircular outlines of permineralized common sheaths of aggregates of coccoidal cells forming rare, short chains (arrowed), transmitted light; *f*, a colony of the extant *Entophysalis samoensis* Wille built of *Gloeocapsa*-like aggregates of cells (*g*) enclosed in common sheaths (after Wille).



latter, which results in their higher transparency and indistinct outlines. Granular microfabric is common in stromatoporooids and has been referred to as cellular, melanospheric or maculate microstructure¹⁵ (Fig. 1b and c).

The spheroidal bodies composing the infillings of astrophorizae can be identified as remnants of permineralised aggregates of cells of coccoid cyanophytes. Identical structures have been observed in modern calcareous stromatolites formed by entophysalidacean blue-green algae^{16,17}, where they form colonies composed of numbers of cells arranged in pseudofilamentous strands (*Entophysalis*) or are distributed irregularly^{18,19}. Such cells, surrounded by mucilage sheaths, are usually found within greatly enlarged common mucilage sheaths enclosing *Gloeocapsa*-like aggregates (Fig. 2f, g). It has been only recently observed¹⁷ that during permineralisation with calcium carbonate it is the tough outer sheaths which envelop particular aggregates of cells that are most resistant, and commonly remain as the only trace of the primary structure of a colony. Similarly, resistant sheath material has been observed in Late Precambrian pleurocapsalean cyanophytes from silicified dolomite²⁰. The filamentous pattern of astrophorizal canals is reminiscent of linear cell masses of some siphononematacean cyanophytes during the stigonematoides growth stage²¹. The granular microfabric of the astrophorizae and the surrounding skeleton indicates that the same or very similar cell aggregates took part in the formation of both.

There are two questions related to the last statement: first, why, having the same microstructural character as the surrounding skeleton, the astrophorizal infilling is darker and less crystalline, and second why the astrophorizae are usually found empty? An explanation to the first question may lie in the colony organisation of extant coccoid cyanophytes in which *in situ* germination of gonidia, endo- or exospores or even groups of nannocytes leads to the formation of new colonies continuing growth as an integral part of the old colonies^{18,19,22}. *In situ* germination of spores is particularly common in some pleurocapsalean cyanophytes²⁰. There is little doubt that astrophorizae represent such *in situ* developed new coccoid colonies, but with significantly reduced permineralisation potential compared with the parent colony. The modern counterpart of the stellate pattern of astrophorizal canals can be found in radially filamentous juvenile stages of such colonial coccoid cyanophytes as *Oncobrysa rivularis* or *Pleurocapsa minor*²¹. As in astrophorizae, the filamentous aggregates of cells in these forms grow first more or less parallel to the substratum branching dichotomously or trichotomously and then, as a result of tetrachotomy, grow upwards losing their filamentous habit. Empty astrophorizal canals represent weak or absent permineralisation of the young colonies, insufficient to protect the organic matter against decomposition. Differences in the degree of permineralisation of different growth stages within the same coccoid strains means active control of the mineral precipitation at least by the young colony. More detailed studies on the organisation and mode of formation of modern coccoid cyanophytes, especially those encrusted by calcium carbonate, are needed before a complete explanation of stromatoporoid biology can be given.

Removal of the Stromatoporoidea from the animal kingdom to the Cyanophyta requires the complete re-evaluation of their taxonomy, palaeobiology and geological significance. This and a more extensive discussion of the new data presented here will follow in a forthcoming paper. The main conclusions are:

(1) stromatoporoids represent permineralised colonies of coccoid cyanophytes closely related to those associated with fossil and modern calcareous stromatolites (cyanophytic stromatoids), formed by *in situ* precipitation of calcium carbonate,

(2) stromatoporoid stromatolites are the Phanerozoic continuation of the Precambrian calcareous stromatolites in

which coccoid communities also had a considerable role^{23,24}.

(3) stromatoporoid stromatolites with their internally differentiated structure, and clear evolutionary tendencies⁴ may contribute significantly to the systematics and correlative value of Precambrian stromatolites,

(4) the very high permineralisation of stromatoporoid coccoid cyanophytes is comparable only with calcium carbonate encrusted cyanophycean communities known from modern freshwater and brackish environments²⁵. This suggests that the epicontinental seas occupied by stromatoporoids had other than present-day salinities, a conclusion supported by the recently reported²⁶ *in situ* association of *Amphipora* with non-marine volvocacean algae.

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Palaeognathous bird from the Cretaceous of Central Asia

Two skulls of an unquestionably palaeognathous bird were found by the Polish–Mongolian Palaeontological Expedition to the Gobi Desert¹. Both specimens originate from the same locality, the beds being referred to as Barun Goyot Formation (?Campanian²). One skull, badly damaged, has been described as *Gobipteryx minuta* and the new order Gobipterygiformes has been allocated tentatively to the Palaeognathae³. Examination of the second, better preserved, although incomplete skull has now confirmed this allocation. Thus, *Gobipteryx* is the oldest known palaeognathous bird, the earliest hitherto recorded fossils of this group being of Eocene age⁴. I estimate the skull of *Gobipteryx* to be 45 mm long, so that the bird was roughly the size of a partridge.

Gobipteryx displays palaeognathous affinities both in the pattern of the palate and the features directly related to the ratite rhynchokinesis⁵ (Figs 1 and 2). Anteriorly the vomers are fused and closely approached by the maxillopalatines. At the rear, the vomers diverge gradually and articulate suturally with the pterygoids, which have a medial socket near the posterior end, obvious for a basipterygoid articulation. The palatine articulates suturally with the outer margin of the pterygoid, so that parasphenoidal contact is ruled out. As far as it preserved, the palate of *Gobipteryx* satisfies all the criteria of the palaeognathous type⁶. The most

striking rhynchokinetic feature is the anterior extension of the nasal opening together with the lack of the connection between the nasal and the maxilla. The free-ending, ascending process of the maxillopalatine, present in *Gobipteryx*, elsewhere occurs only in some ratites (*Rhea*, *Casuarius* and *Dromaius*). Moreover, the loose contact between the nasal process of the premaxilla and the premaxillary process of the nasal, clearly seen in *Gobipteryx*, occurs in all ratites and some members of the Tinamidae. The common occurrence of such a palatal and rhynchokinetic condition provides new evidence of the coupling of these two characteristics⁶.

In some features *Gobipteryx* seems to resemble the Australian ratites, in particular its palate structure is similar to that of *Casuarius*: the posterior bifurcation of the vomers is very deep, with their lateral edges lying more ventrally than the medial ones; the pterygoids widen distinctly in the region of the palatine articulation; the palatine articulates mostly if not exclusively with the pterygoid; the outer margin of the palatine is much thicker than the inner blade and projects ventrally. In *Gobipteryx* the palatine is posteriorly enclosed in the slight anterior bifurcation of the pterygoid, with the small outer tine projecting antero-laterad. Such a pterygoid bifurcation was thought to be unique in *Apteryx*⁷. Thus, *Gobipteryx* shares some characteristics with both *Casuarius* and *Apteryx*⁸ and unless it is

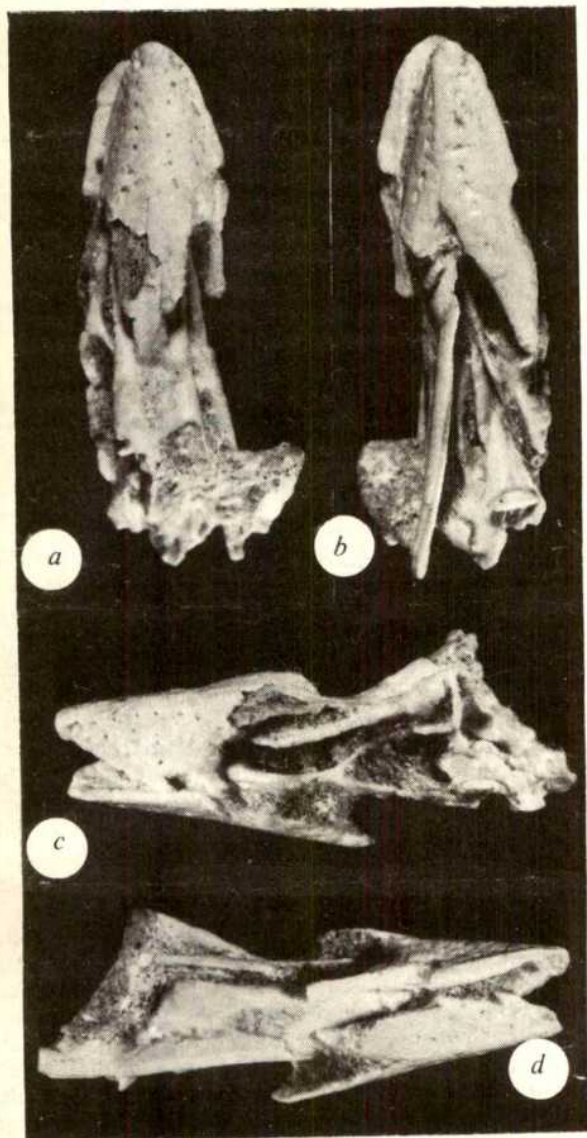


Fig. 1 *Gobipteryx minuta* Elzanowski 1974. The second skull. a, Dorsal view; b, ventral view; c, left lateral view; d, right lateral view ($\times 2.5$).

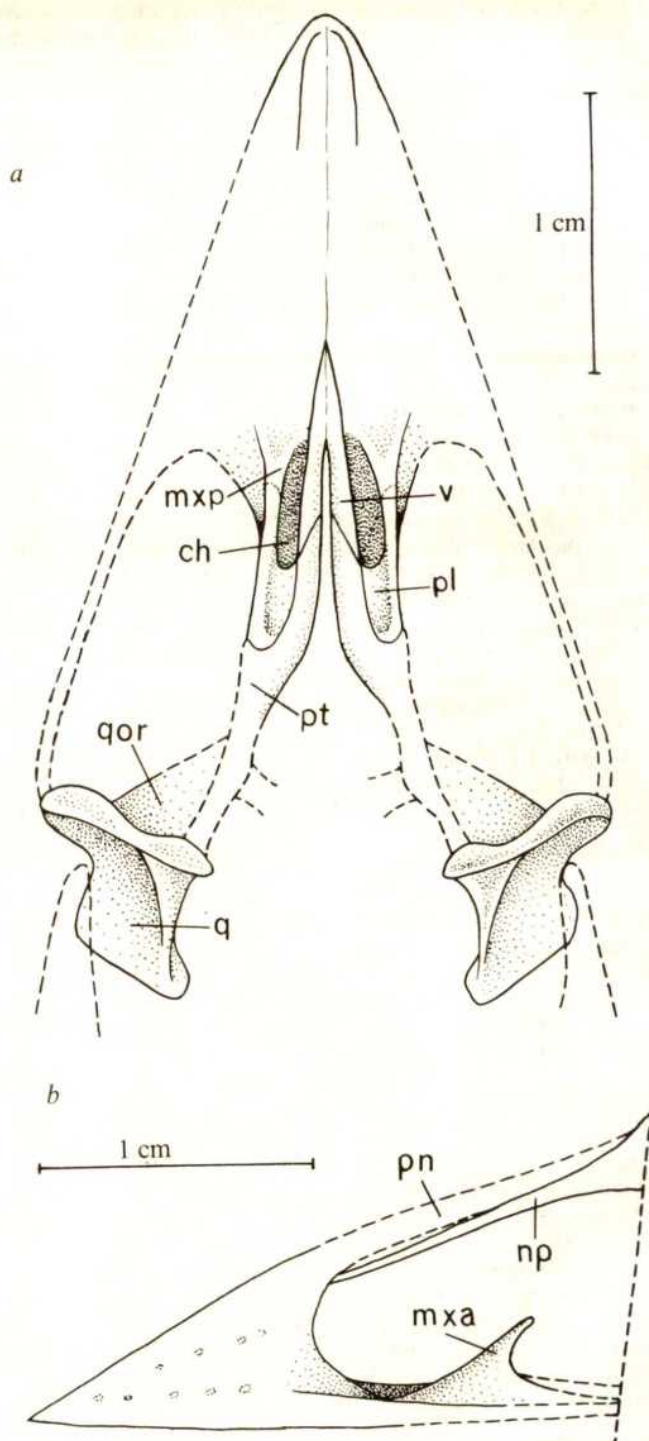


Fig. 2 *Gobipteryx minuta* Elzanowski 1974. Partial restorations of the skull. a, Ventral view; b, rostral part in lateral view. ch, internal naris; mxa, ascending process of the maxillopalatine; mxp, maxillopalatine; np, premaxillary process of the nasal; pl, palatine; pn, nasal process of the premaxilla; pt, pterygoid; q, quadrate; qor, orbital process of the quadrate; v, vomer.

assumed that these are primitive characteristics of the Palaeognathae as a whole, they might indicate a closer relationship between the Casuariformes and Apterygiformes, as suggested already^{8,9} though later challenged¹⁰.

The mandibular articulation of the *Gobipteryx* quadrate is atypical of the Palaeognathae, and resembles that of the pigeons: it is bicondylar without any structure corresponding to the posterior condyle. Such a quadrate is also clearly similar to that found in some smaller theropods, such as *Gallimimus*¹¹ or *Dromaeosaurus*¹², with the orbital process corresponding well to the pterygoid flange of the dinosaurs.

Palaeognathous birds are commonly thought to be of southern origin¹³⁻¹⁵, although this view was challenged for struthionids and aepyornithids¹⁶. Moreover, the dispersal of palaeognathous birds between the Old and New Worlds is explained only by the Gondwanian connections¹³. The Asiatic origin of *Gobipteryx*, the oldest known representative of the group, gives some evidence in favour of the northern dispersal. If migration across Beringia was possible for small mammals¹⁷, it would be the more so for birds although the flying ability of *Gobipteryx* remains a vexing problem.

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Rivalrous texture stereograms

THE two eyes receive slightly differing views of the world and it has been known since the invention of the stereoscope, 140 yr ago, that these slight differences are utilised by the visual system for stereopsis—the perception of the relative depths of objects on the basis of binocular disparity. The central theoretical problem in stereopsis is simply stated: how is disparity information extracted from the two retinal images? We examine here various possible answers to this question.

The most obvious possibility, and historically the first to be considered, is that the brain identifies objects in each eye's image, notes their different relative locations and computes stereopsis accordingly. But a monocular object recognition stage is emphatically not a requirement for stereopsis. This has been clearly demonstrated by the random-dot stereograms of Julesz¹. These stimuli show that stereopsis can be perceived even when the two eyes receive

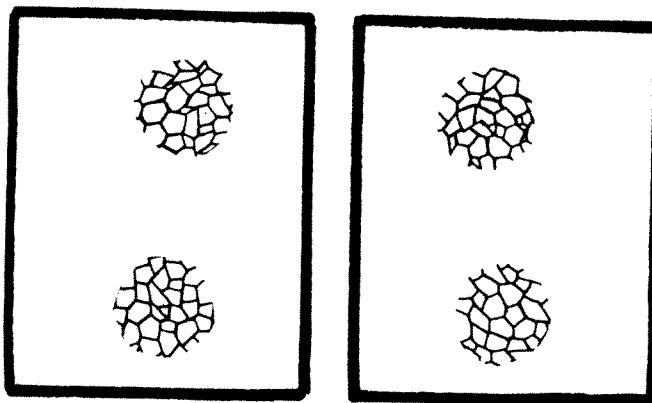


Fig. 1 Rivalrous texture stereogram (after Tausch²). Binocular fusion of the two halves of this figure results in the perception of relative depth (stereopsis) between two circular patches.

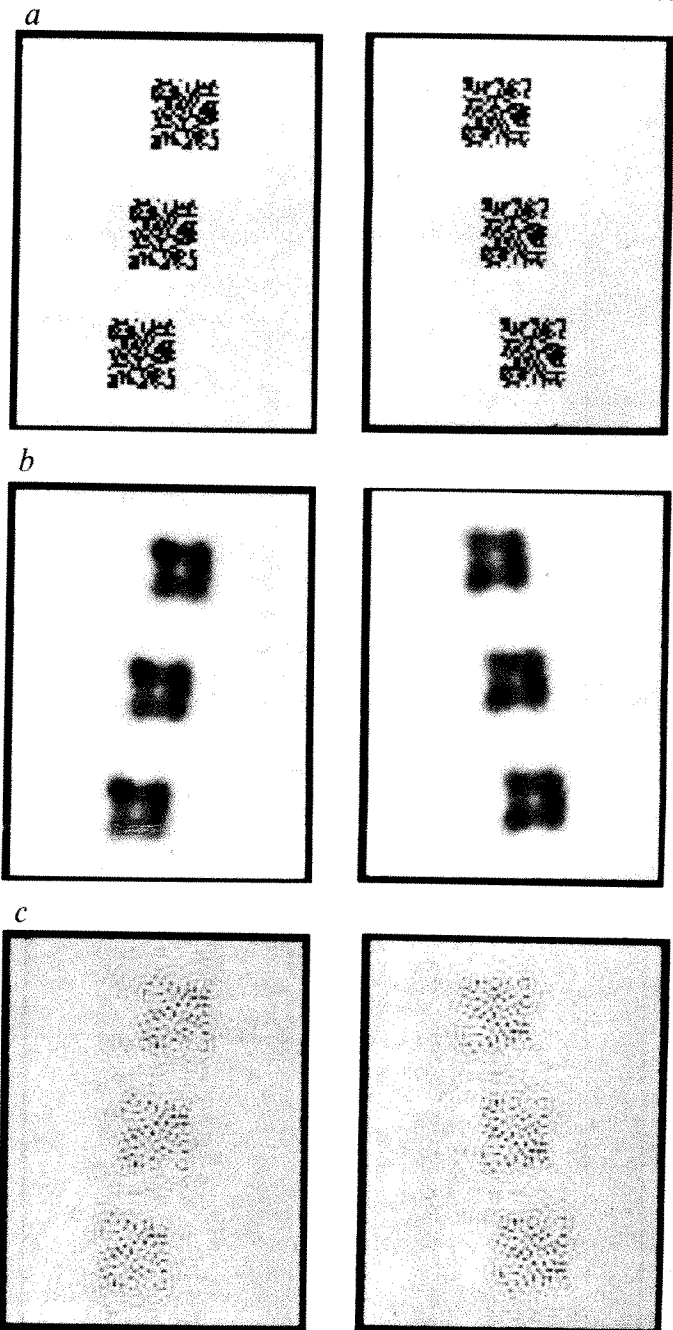


Fig. 2 *a*, Rivalrous texture stereogram in which three squares are seen to lie in different depth planes after binocular fusion although their textures are rivalrous. Each texture is an upside-down mirror image of the corresponding texture in the other field. This renders each pair of textures effectively random as far as binocular fusion is concerned, and hence rivalrous, and yet ensures that the left and right images have identical power spectra. The central squares have zero disparity with respect to the outer frame whereas the upper and lower squares have crossed and uncrossed disparities respectively. Presenting three squares in this way facilitates judgements of stereopsis, particularly in the relatively difficult circumstances of rivalrous texture stereograms, because if stereopsis is present not only do the squares appear in different depth planes but they also tend to appear vertically aligned. It is a methodological improvement over single-square figures³, in which it is not always easy to know whether the segregation of the square from its ground is presenting a masking depth cue which gives a depth impression independently of stereopsis. *b*, Low pass filtered version of (*a*) which contains only spatial frequencies below 1.0 cycle per degree if viewed from five times picture height. *c*, High pass filtered version of *a* containing only spatial frequencies above 4.0 cycles per degree. The original stereograms were prepared using a full-tone facsimile picture receiver monitored with a UDT photometer and viewed with back illumination. Photographic and printing processes inevitably introduce some spectral distortion from the originals but the various stereo effects which can be observed are faithful nonetheless.

random textures in which are embedded disparate forms completely hidden to monocular view but which nonetheless appear in depth due to the fact that their random textures are correlated in the two fields. This finding has led Julesz to conclude that there must exist stereopsis mechanisms which operate by effecting a 'low level' point-for-point matching of the two retinal images.

Given then that monocular object recognition is not a necessary precursor of stereopsis, it is interesting to ask whether it is a sufficient precondition. Consider Fig. 1, for example. This 'rivalrous texture stereogram'² successfully presents disparity depth cues by means of 'object' information even though there is only random correspondence between the left and right images of these objects at the level of fine texture elements. It is tempting to conclude from this demonstration that, as a 100% point-for-point matching of the left and right images of the objects is clearly impossible, a stereopsis mechanism sensitive simply to monocularly-discriminable shapes, irrespective of their precise detailed textures, must be at work and that therefore the monocular object recognition of disparate objects is indeed a sufficient basis for stereopsis³.

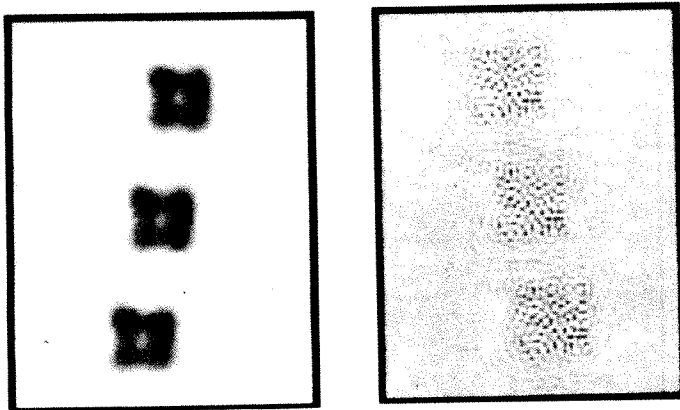
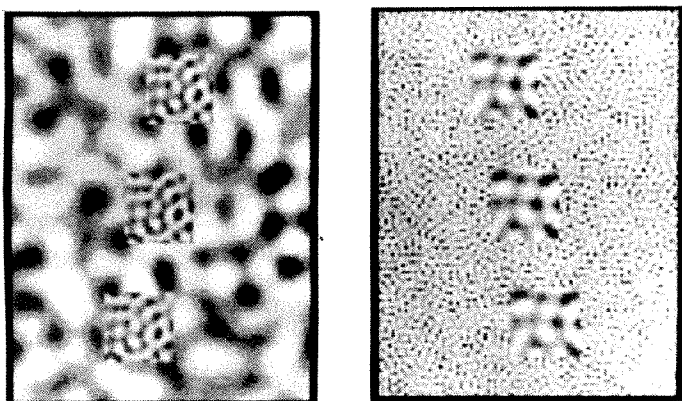


Fig. 3 Stereogram comprised of the left half of Fig. 2b and the right half of Fig. 2c. Stereopsis is impossible.

One conceivable way of accounting for the stereopsis of Fig. 1 in point-for-point terms, however, is to suppose that point-for-point matching follows a stage of spatial frequency filtering. Thus the disparity information carried by the objects might be extracted by point-for-point matching in a 'stereopsis channel' tuned to low spatial frequencies; while at the same time other stereopsis channels tuned to higher spatial frequencies, and thus sensitive to fine texture details, are in states of rivalry. This possibility is supported

Fig. 4 Stereogram comprised of random textures with no overlapping spectral content. Right hand: squares, 1.33 to 1.75 cycles per degree; surround, 4.0 to 5.25 cycles per degree. Left hand: squares, 2.3 to 3 cycles per degree, surround, less than 1 cycle per degree. Stereopsis is impossible.



by the demonstrations of Julesz and Miller⁴ which show that in random-dot stereograms depth can indeed be mediated by one spatial-frequency-tuned channel while others remain in rivalry.

We have tested this explanation using low and high bandpass filtered versions of the rivalrous texture stereogram shown in Fig. 2a. Figure 2b (low pass) and c (high pass) would selectively stimulate low and high spatial-frequency-tuned channels, respectively. As predicted, stereopsis is clearly present in the low bandpass filtered stimulus. Stereopsis, however, can also be established without difficulty in the high bandpass stereogram. This result shows that the idea of only low spatial-frequency-tuned channels being capable of mediating the depth seen in rivalrous texture stereograms is false: such channels would

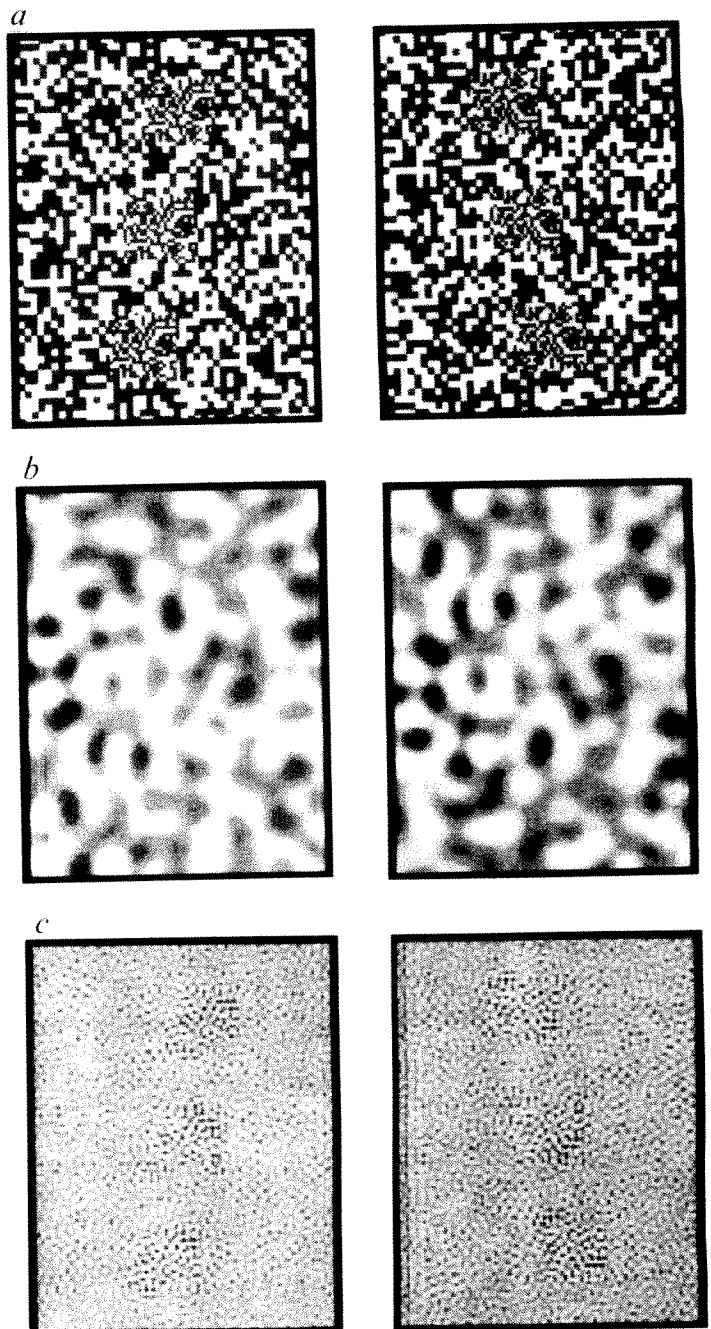


Fig. 5 a, Stereogram in which both squares and their surround are comprised of rivalrous textures (after Ramachandran *et al.*⁵). Rivalrous textures have been created as for Fig. 2. b, Low pass (1 cycle per degree) version of (a). c, High pass (4 cycles per degree) version of (a). Stereopsis is possible in (a) and (c) but with greater difficulty than in Fig. 2c.

not be active in the case of Fig. 2c and yet stereopsis is present.

The fact that stereopsis can be perceived in Fig. 2c suggests once again the operation of a 'monocular-object' stereopsis mechanism which is picking out the monocularly-visible texture boundaries of the squares and ignoring the fact that the details of the left and right textures do not match. It is therefore instructive to ask whether it is possible to obtain stereopsis by combining the left half of Fig. 2b with the right half of Fig. 2c. The squares in each of these half stereograms are of course perfectly visible monocularly and if there existed a mechanism capable of being driven by gestalts formed by texture contours³, then such a combination should surely produce stereopsis. Figure 3 represents this combination and we find stereopsis impossible. The two fields are extremely rivalrous. This result suggests that stereopsis can be obtained from rivalrous texture stereograms only if there is spectral overlap in the two halves of the stereogram, irrespective of the monocular visibility of the texture contours themselves. This conclusion is further supported by Fig. 4, which presents squares composed of rather less markedly different spatial frequencies in the two fields than Fig. 3 and sets them in surrounds also differing in spatial frequency. Again stereopsis is impossible, in spite of the existence of clearly discriminable and disparate monocular gestalts.

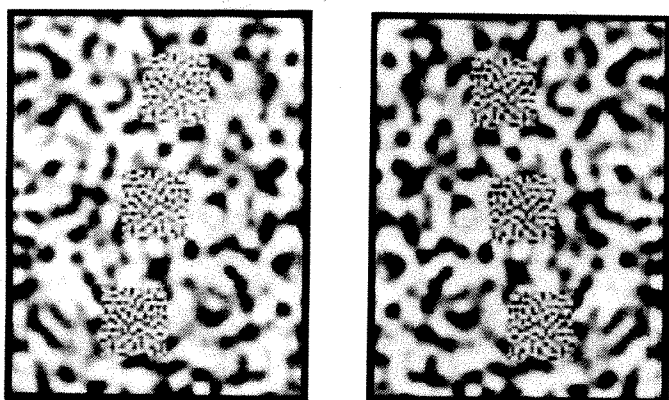


Fig. 6 Rivalrous texture stereogram in which the squares are comprised of spatial frequencies above 3.5 cycles per degree and in which the surround is comprised of spatial frequencies below 1.75 cycles per degree when viewed from five times picture height. Stereopsis is easy to obtain.

Figures 3 and 4 suggest that the notion of spatial-frequency-tuned stereopsis channels is valuable when considering rivalrous texture stereograms. But if such channels are to explain the stereopsis of Fig. 2c, and these channels are to be considered as involved in a process of point-for-point matching, then an account must be offered of how such channels could output stereopsis for Fig. 2c even though the textures of its squares have only random correspondence.

We submit that Fig. 2c has, for a high spatial frequency channel, what might be called partial point-for-point correspondence, rather than no correspondence whatsoever. First, note that the almost uniformly grey (d.c.) surrounds to the squares in Fig. 2c would not produce above-threshold activity in high spatial-frequency-tuned units. An array composed of such units would, quite simply, be 'blind' to these surround areas and not transmit forward any information about bright or dark points for point-for-point stereopsis processing. (The fact that our original high spatial frequency filtering produced these grey surrounds could be misleading here. If Fig. 2c is considered not as a stimulus but as a representation of activity in a high spatial-

frequency-tuned channel when stimulated by Fig. 2a, then it must be remembered that the grey level in this representation is the baseline or noise activity level, with above threshold departures from this level, bright or dark, being the only transmitted information to later stages of processing. That is, the white points in Fig. 2c can be regarded as reflecting above-threshold activity in high spatial-frequency-tuned neural units with on-centre/off-surround receptive field profiles, the black points as above-threshold activity in similar units with off-centre/on-surround profiles and the grey points as below-threshold activity in both these kinds of units.) Second, consider the possible matches which could be established for brightness points lying within the 'disparate' areas of the squares (that is, those areas 'confronted' with d.c. surround in the other field of view). These points could find no matches whatsoever at non-disparate locations, simply because the d.c. would not produce any above-threshold activity at these locations. If they are to find a match at all it would have to be a disparate one. And the possibility arises that a sufficiently large number of such matches occurs for stereopsis to be generated. Of course, there will never be a state of perfect point-for-point matching obtained for these areas, due to the inherently random selection of the two textures forming the squares. Nonetheless, a state of partial point-for-point correspondence does obtain and, as Julesz¹ (pages 271-275) has amply demonstrated, partial correspondence is an adequate basis for stereopsis. Moreover, there is no reason, in principle, why a point-for-point mechanism should require perfect correspondence as a *sine qua non* of its successful operation.

We report elsewhere³ a more detailed exposition of this idea and a development of Julesz's dipole model of stereopsis¹ which would be capable of providing the hypothesised binocular matching. At present we simply note some evidence which supports the idea in general terms.

Our explanation of the stereopsis of Fig. 2c depends crucially on there being no matches available in the surround areas. This leads naturally to the following question: what would happen if both the squares and their surrounds were composed of rivalrous textures? Such stereograms have already been devised³ and an example is shown in Fig. 5a. The squares of this stereogram are discriminable simply by virtue of the fact that their texture elements are half the size of those in the surround. Stereopsis from this stereogram is poor and unstable, as the inventors of this stereogram note and as the reader may judge. It is certainly of greatly reduced quality compared with the stereopsis from Fig. 2a-c. Figure 5b and c presents respectively low and high pass versions of Fig. 5a. As would be expected, the squares do not exist as monocularly-discriminable entities in either half of Fig. 5b. Fusion of this stereogram usually results in complete rivalry although we find that some observers occasionally report depth impressions relating to certain blobs which happen, through the random processes of manufacture, to have *ad hoc* and fortuitous disparities. In Fig. 5c the squares do exist as monocular entities although in a much attenuated form when compared with the squares in Fig. 2c. We find that stereopsis from this figure is possible although it is not obtainable as readily as in Fig. 2c, nor is it as stable. These impairments, caused by including a rivalrous surround, are exactly what would be expected given our general interpretation because local processes serving the squares in Fig. 5c can now obtain a better degree of matching with their surrounds than can the squares of Fig. 2c. Of course, given that the squares in Fig. 5c are still characterised by slight brightness differences from their surrounds, one would still predict that stereopsis should be possible because square/square matches will have a slight edge over square/surround matches. Nonetheless, because the brightness differences characterising the squares are not very marked, this superiority would not be as great as in Fig. 2c—hence the

fact that stereopsis can be obtained in Fig. 5c but in an impaired form.

One interesting prediction that can be drawn from our analysis of Fig. 5a is that stereopsis should be clear and easy to obtain from stereograms with rivalrous texture surrounds if the spectral content of the surround is sufficiently different from that of the squares. In such cases, the spatial frequency tuning of the channel dealing with the squares will result in this channel effectively dealing with a uniformly grey surround (as in Fig. 2b and c). Figure 6 presents a stereogram of this type and it produces clear stereopsis, as predicted.

The important conclusion deriving from our analysis is that stereopsis in rivalrous texture stereograms can be explained by spatial-frequency-tuned stereopsis channels without any need to involve more complex stereopsis mechanisms incorporating monocular object recognition.

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Role of stimulus environment and duration of processing in long term memory

A MAJOR concern of current memory research is to identify how information is encoded and transferred from short term memory (STM) to long term memory (LTM)^{1,2}. It has been reasoned that while an item remains in STM, it can be maintained through rehearsal; as a consequence, the corresponding LTM trace is built up. Thus, one may postulate that the adequacy of an item's registration in LTM is a positive function of the length of its stay in STM. Such a mechanical and simplistic account of memory has been challenged by a series of experiments which showed that when short term storage times were measured, these times did not predict long term retention^{3,4}. We report here an experiment which manipulated the processing time as well as the processing context and showed that the processing time had differential effects, sometimes beneficial, sometimes harmful, and sometimes neutral, on an item's long term retention, depending in which type of processing context the item was embedded. Such results argue for a perceiver-environment interactive theory of memory.

To minimise the difference between subjects' mnemonic strategies, the present experiment used an incidental-learning paradigm—that is, the subjects were not informed about the recall test until the very end of the experiment. Instead, they were told that the experimental task was a target-search task and were made to believe that our interests were the accuracy and the speed of their search. In fact, the experimental task was similar to the visual search task used by Neisser and Beller⁵ except for the final unexpected free recall test. In general, the subject was required to hold a target word in his memory and then to scan through a search list until the particular target word was found. By manipulating the target position within the search list and the nature of others in the list, we were able to vary the processing duration as well as the stimulus environment. In the present study, there were four different list structures and three different target positions, forming

a 4 × 3 factorial design with both variables as within-subjects measures.

The four list structures were made up in the following ways. To illustrate, let the target word be TABLE. For the phonemic-similar (PS) condition, all other list words were phonemically similar to the target—for example, STABLE, CABLE, MAPLE. For the semantic-similar (SS) condition, all other list words belong to the same semantic category as the target word—for example, CHAIR, SOFA. For the random (RAN) condition, all other list words were randomly selected from the Thorndike-Lorge's high frequency words. Finally, for the nonsense (NON) condition, all other list items were nonsense but pronounceable letter strings with length ranging from three to five. The target words were always embedded in list position 3, 9 or 15. The search lists were always 20 items typed (double spaced) vertically in a column. The subjects were 40 undergraduate students enrolled in an introductory psychology course at the University of California (Riverside), and were tested individually.

The subject was instructed to search the list from the top to the bottom, using a pencil to direct his attention to each word. The subject was instructed to search for the target word as quickly and as accurately as possible, and to place a check beside the target word as soon as it was found. The experimenter recorded the search time for every word with a stop watch. Two practice trials with numbers preceded the experimental task of 12 target searching trials. After the last searching trial, the subject was asked to fill in a questionnaire which included some autobiographical data—for example, name, address, phone number, and so on, for about 2 min. An unexpected recall test was then administered. The subject was asked to write down as many target words as he could in any order he preferred. This recall interval was fixed at 2 min. A post-experimental interview revealed that no subject had anticipated such a "tricky" test.

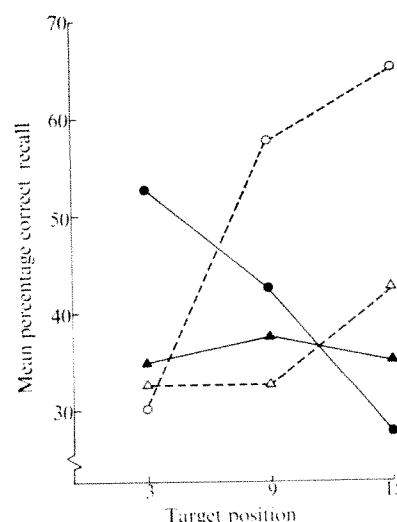


Fig. 1 The subject was asked to hold a target word in his memory and then to scan through a list of words until that particular target word was found. At the end of 12 such visual search trials with 12 different target words, an unexpected recall test for all the target words was administered. Mean percentage recall of the target words was plotted in this figure as a function of the processing time and the processing context. The length of the processing time was manipulated by placing the target word at one of the three positions (that is, 3, 9 and 15) within the search list. The processing context was manipulated by varying the nature of the search list: ●, all other words in the search list were phonemically similar (PS) to the target word; ▲, all other words in the search list were semantically similar (SS) to the target word; ■, all other words in the search list had no apparent relation to the target word (RAN); ◆, all other items in the search list were non-words (NON) made up with nonsense syllables.

The results of the experiment are summarised in Fig. 1. Mean percentages of correct recall in various list conditions were plotted as a function of the target position. It is clear that the duration of processing has differential effects on the item's long term retention, depending on the stimulus environment in the processor at the time of processing the item. Statistical tests (ANOVA) substantiated the interaction between target position and list condition ($F(6, 234)=4.78, P<0.01$).

The first striking aspect of the data depicted in Fig. 1 is the fact that when the target was embedded at position 3, the PS list condition produced the best recall performance compared with the other three conditions (all P values <0.05 with Tukey's test) which did not differ from one another. This is a very intriguing finding. Most current memory theories would predict that since the item would be better retained in a non-interfering environment than in an interfering context, recall performance would be worse in the PS condition where the processor is filled with acoustically similar items. But the data showed that this prediction holds only at position 15. The relative superiority of the PS condition at positions 3 and 9 is difficult to account for by current memory theories.

From the viewpoint of a perceiver-environment interactive model, however, the superiority of the PS condition at position 3, followed by a gradual decline at position 9 and 15, can be readily explained. According to Neisser and Beller⁵, in a search task the subject needs only to hold a phonemic representation of the target word while he is searching the list. The immediate match between the temporarily held representation and the acoustically similar environment pushes the recall level up about 20%. This facilitating effect of a phonemically similar environment suggests that an acoustically similar sound helps to confirm and establish a retrieval cue which is phonemic in nature. At position 3, the newly established phonemic cue is relatively unique and distinct. As there are more acoustically similar items to be searched, however, this memory code becomes less unique and less distinct due either to storage deterioration (an 'acid-bath' hypothesis)⁶ or to a retrieval confusion (a 'cue-overloading' effect)⁷. Thus, in the PS condition, short processing (but long enough to have three or four items processed) has a positive effect on the item's retention. Once the phonemic code is established, however, the longer the processing time, the more items will be checked into the processor. Subsequently, prolonging the processing time has a negative effect on item's retention.

When the target word was embedded in an environment which bears no apparent relationship to the word itself, such as in the RAN and the NON conditions, the duration had no effect on the item's retention. For no matter how long the item has been held in the processor, there will be little, if any, possibility of interaction between the item and its environment.

When the item was embedded in a semantically related context, the results showed quite a different pattern. The phonemically encoded representation of the target word did not match the environmental context. In fact, the environment contained information related to the semantic rather than the phonemic aspect of the target word. The results of position 3 suggest that only two semantically related items are not enough to activate a semantic code for the target item. Thus, the SS condition functioned like the RAN and NON conditions in that recall was not facilitated by the environmental context. The results at positions 9 and 15 suggest that when the number of semantically related words searched is large enough to activate a semantic code, the recall probability is suddenly boosted and continues to grow from position 9 to 15. Thus, when the initial representation does not match its environment which contains some useful information by itself, increasing the processing time increases the possibility of discovering

that information. In these conditions, duration of processing does have a positive effect on the item's retention.

The pattern of results from the present study suggests that the effect of processing duration on item retention is a very complicated matter. An item is not processed in a vacuum; rather, the processor is always shared by other ongoing activities which become a functional environment for the processed item. To the extent that the environment does contain some information which will help the processor to establish a more durable memory code, processing time will be beneficial. When the environment contains no systematic information to help the processor to establish a unique and distinct code, time itself is not an important factor.

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Relationship between reward-enhancing and stereotypical effects of psychomotor stimulant drugs

BLEULER has described stereotyped behaviour as "one of the most striking external manifestations of schizophrenia"¹. Schizophrenic stereotyped behaviour has been found in the spheres of movement, action, posture, speech, writing, thought and desire¹. Abuse of the psychomotor stimulant drugs, such as the amphetamines, methylphenidate, cocaine and pipradrol, can cause a psychosis which is clinically indistinguishable from paranoid schizophrenia², and which contains stereotyped components^{3,4}. In animals, acute doses of the stimulants can induce stereotyped behaviour which is apparently under minimal situational control, and which can disrupt normal activity⁴. In contrast, low doses of the stimulants can apparently improve learning and performance in a variety of situations, in both animals⁵ and man⁶. This paper demonstrates a relationship between these two apparently diverse actions.

The facilitation of behaviour by stimulants has been attributed to the enhancement of the effects of rewarding stimuli, by the potentiation of a brain reinforcement mechanism⁷. One of the major functions of reinforcement is to effect learning, an associative process, and it is possible for neutral stimuli, which are subsequently paired with primary rewards, such as food or water, to acquire reinforcing properties. These stimuli are termed conditioned reinforcers (CRs), and there is some evidence that stimulants can augment the effects of conditioned reinforcement^{8,9}, thus supporting the reward-enhancement hypothesis. It is, however, unclear how this hypothesis, or any other that has been advanced to explain the behavioural effects of stimulants^{10,11}, can account for the stereotyped behaviours produced by these drugs. This paper will show that stimuli with acquired motivational significance (CRs) can be crucial determinants of the selection of responses which form part of a stereotyped pattern of behaviour. Experiment 1 shows

that a dose of 10 mg kg^{-1} of the stimulant drug pipradrol, can facilitate the learning of a novel spatial discrimination task reinforced solely by a stimulus formerly correlated with reward. Experiment 2 demonstrates that although CR stimuli are important in the selection of the responses which are stimulated by pipradrol, these responses are performed in a perseverative manner, being part of a stereotyped pattern of behaviour induced by the drug.

Experiment 1 consisted of two stages. In stage 1, rats were trained either to associate, or not to associate, a specific stimulus with the presentation of water. Stage 2 tested the strength of the association formed in training, by using the stimulus as a reinforcer to sustain new learning, a stringent criterion for the establishment of conditioned reinforcement¹². Male albino rats (150–160 g at commencement of training) were provided with food *ad libitum* and subjected to a 23-h water deprivation schedule throughout the experiment. Initially, they were trained to push a panel in a standard Skinner box to collect water from an elevated dipper⁸. During stage 1, the dipper operated for 7.5-s periods, at intervals of 30 s for a total of 30 reinforcements per session, for two sessions. On three further sessions, water rewards were programmed to occur at variable intervals, averaging 30 s.

There were two conditions, CR and FS, which used different groups of rats. In the CR condition ($n=14$), a light preceded each presentation of water by 0.5 s, remaining switched on for the duration of the reinforcement. It was thus perfectly correlated with reward and was the CR stimulus. In the FS condition ($n=14$), the same light was presented according to a similar schedule, but it was randomly correlated with reward. It therefore had no associative relationship with reward and was merely a familiar stimulus (FS). There were two levers in the box during training, situated to the left or the right of, and equidistant from, the panel. Responding on either lever had no consequence for the animal in stage 1.

For stage 2, there were three 30-min sessions, each separated by 48 h to allow recovery from drug effects. No rewards of water were given during these three sessions. Animals were divided into equal drug and control groups (both $n=7$) within the CR and FS conditions. The drug groups received intraperitoneal injections of 10 mg kg^{-1} pipradrol, 15 min before each of the 30-min sessions. This dose was used since it has been found to be effective for studying the potentiation of CR by the drug in another paradigm⁹. In addition, the purpose of this experiment was to manipulate behavioural variables, while keeping pharmacological ones fixed. The control groups received injections of the drug vehicle (1:2 mixture of polyethylene glycol and distilled water). In both conditions, CR and FS, pressing one of the two levers in the box now produced the light stimulus with $P=0.5$, whereas responding on the other lever had no programmed consequence. The lever providing the stimulus was counterbalanced over subjects. This constituted a novel spatial discrimination task, reinforced solely by the light stimulus. Levers were designated as CR or NCR (no CR) or alternately as FS or NFS (no FS), in the two conditions. Responses on each lever, panel pushes and light presentations were totalled and recorded at 15 min interval during the session.

The results obtained strongly support the hypothesis that the stimulation of responding by pipradrol was controlled by the CR stimulus. An analysis of variance was carried out on the lever press totals for each rat, in the drug and control groups of the CR and FS conditions. All data were subjected to a square-root transformation to preserve homogeneity of variance¹³. The analysis revealed a significant interaction of drug with condition with the lever ($F=9.10$, d.f.=1,24; $P<0.01$), which represents the highly specific action of pipradrol illustrated in Fig. 1. Responding was greatly elevated on the CR lever in the pipradrol group,

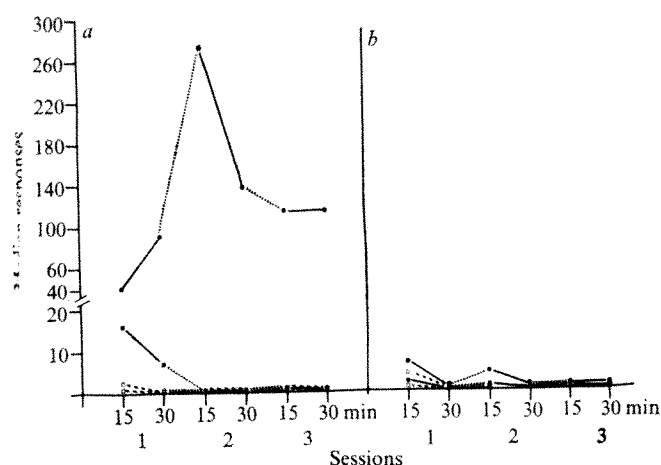


Fig. 1 Experiment 1: Median response totals in the CR (a) and FS (b) conditions for drug and control groups, for different levers, plotted at 15- and 30-min stages for each of the three 30-min sessions in stage 2. The use of medians to express these data is preferred, because it provides a more accurate description of the group data. The use of means would greatly inflate the scores for the drug group in the CR condition due to the contributions by certain rats, of very high response totals on the CR lever. The dotted lines between sessions indicate that these are not continuous functions of time. Note the change of scale on the vertical axis, to accommodate the low response totals of the control conditions. a: Pipradrol 10 mg kg^{-1} : ●, CR; ■, NCR; Control: ○, CR; □, NCR. b: Pipradrol 10 mg kg^{-1} : ●, FS; ■, NFS; Control: ○, FS; □, NFS.

although responding on the NCR lever quickly reached control levels and lower. This shows that the stimulant action of pipradrol was restricted to the CR lever and could not therefore have been due to nonspecific increases in responding. Further, the much lower level of responding in the FS condition, for the FS stimulus, shows that the CR condition results are not simply due to the drug enhancing the reinforcing properties of stimulus change¹⁴, but depend on the learned association of the stimulus with primary reward. Pipradrol also apparently enhanced the learning of the spatial discrimination task to obtain CR. Responding for the CR initially increased over time under pipradrol, whereas responding in all other conditions generally showed monotonic decrements. In fact, all seven rats in the pipradrol group responded more for CR on session 2 than on session 1. In addition, five of these seven rats showed increases in responding on the CR lever which were concomitant with decreases in responding on the NCR lever. This pattern is consistent with the interpretation that pipradrol facilitated the learning of the task to obtain CR, and that extinction of responding only began to occur later, as the association between the CR and water became weaker. The experiment emphasises the importance of stimuli of prior motivational significance in determining the selection of responses which are stimulated by pipradrol. It also provides the most convincing evidence to date that stimulant drugs enhance the effects of conditioned reinforcement, especially since the potency of CR to sustain new learning in control organisms is not great^{12,15}, a view supported by the control data of the present experiment.

Observations of the drug-treated rats using closed circuit television revealed that these animals displayed stereotyped patterns of behaviour, which could include repetitive sniffing, head movements, rearing in the vicinity of the CR lever, and more rarely, incipient approach responses to the panel. The important question then arises: are the lever presses, in the patterns of behaviour, goal directed, occurring in spite of the competing stereotyped activity, or do they represent the perseveration of responses that were once goal directed and now form part of a stereotyped pattern of behaviour? The second possibility suggests that pipradrol

exaggerated the differences in responding on the CR and NCR levers in experiment 1, by increasing the tendency to repeat responses. It also suggests that stereotyped behaviour can have learned components.

Experiment 2 tests between these hypotheses by using a task in stage 2 in which perseveration of responding acts against, rather than in favour of, learning. If it is possible to demonstrate that the lever pressing for CR has perseverative properties, then its similarity to other forms of stereotyped activity would be underlined. Stage 1 was similar to that of experiment 1, except that the CR stimulus was 0.5 s long. In stage 2, the CR consisted of this stimulus and the brief (0.3 s) elevation of the empty water dipper. To obtain CR, the learning of a two-component response sequence was required, involving a single response on each lever, made in the precise sequence, component 1, component 2. Thus perseveration of responding on either lever would lead to disruption of learning and a reduction in obtained CRs.

Rats trained as previously were injected with either 10 mg kg⁻¹ pipradrol ($n=6$), or vehicle ($n=6$), 15 min before each of six 30-min sessions. Figure 2 shows that there was a remarkably similar pattern of responding to that shown in experiment 1. There were sharp increases in responding on the second component of the response sequence, concomitant with decreases in responding on the first component, for drug-treated rats. This perseveration of responding led to a progressive reduction in obtained CRs, although the absolute response rate was still increasing.

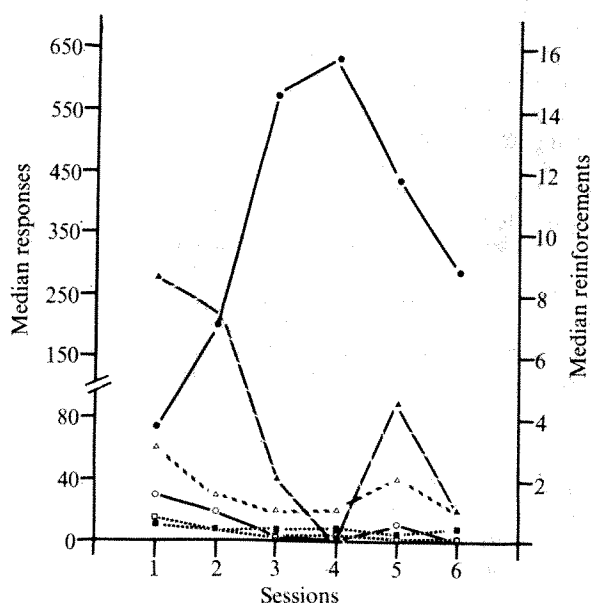


Fig. 2 Experiment 2: Median response totals plotted over the six 30-min sessions of stage 2 for component 1 and component 2 lever press totals. The differential stimulation of component 2 over component 1 in the drug group, as compared with the control group, is significant, following an analysis of variance similar to that used in experiment 1 ($F=6.43$, d.f.=1,10, $P<0.05$). Note the change of scale on the left-hand side vertical axis. Median CR totals are also plotted on the right-hand side vertical axis, as a function of sessions. Pipradrol 10 mg kg⁻¹: ●, component 2; ○, component 1; ▲, reinforcement; control: ■, component 2; □, component 1; △, reinforcement.

There were, in fact, no overall differences in the number of CRs obtained by the drug and control groups (Mann-Whitney U test, $P=0.132$, two-tailed). It is of great importance that the stimulation of responding in the pipradrol group always occurred for the component of the sequence closest in time to the presentation of CR. Although the selection of the response was once again controlled by the CR contingency, however, its performance was perseverative, since it resulted in a reduction of obtained CRs.

Pipradrol thus clearly failed to sustain the learning of the response sequence to obtain CR. The fact that responding was perseverative, and integrated into a stereotyped pattern of behaviour, as in experiment 1, shows, however, that it is probable that stereotyped behaviour can have learned components. It is of course difficult to say whether most forms of stereotypy result from the perseveration of responses formerly goal directed. It is clear that the learned components in the present patterns were not performed indefinitely; extinction of them occurred in most instances, showing that perseveration does not completely override environmental controls. It is interesting, however, that responding was very persistent in certain cases in experiment 2, in which the frequency of CR was low, therefore diminishing the opportunity for extinction of the original association of the CR with water. One rat showed increasing response rates over the six sessions, making more than 3,400 responses on the last session, receiving only one CR. The results from both experiments support the hypothesis that a general action of stimulant drugs is to cause increased repetition of responding, with response selection being dependent in part on environmental contingencies³. Thus the 'reward-enhancing' and stereotyped effects of the psychomotor stimulants are postulated to arise from the action of a common mechanism.

This hypothesis can explain the apparent conditioning of stereotypy in amphetamine-treated cats, as well as the subsequent 'fragmentation' and ritualisation of their responses into stereotyped patterns¹⁶. It can also explain the idiosyncratic nature of the stereotypies of human amphetamine addicts, which involve perseveration of well-established habits such as car maintenance and house cleaning³. Finally, it has implications for the apparently meaningless stereotyped activities of certain psychotic states, such as autism¹⁷ and schizophrenia^{1,2,18}. It is suggested that these activities may arise from a fixation on behaviour which had been initially purposeful. In support of this, Bleuler¹ has described schizophrenic stereotypies which were related to events of significance occurring in the previous history of the subjects. This paper has shown that an analogous mode of behaviour may occur in rats treated with amphetamine-like drugs, and strengthens the use of drug-induced behaviour as model psychoses.

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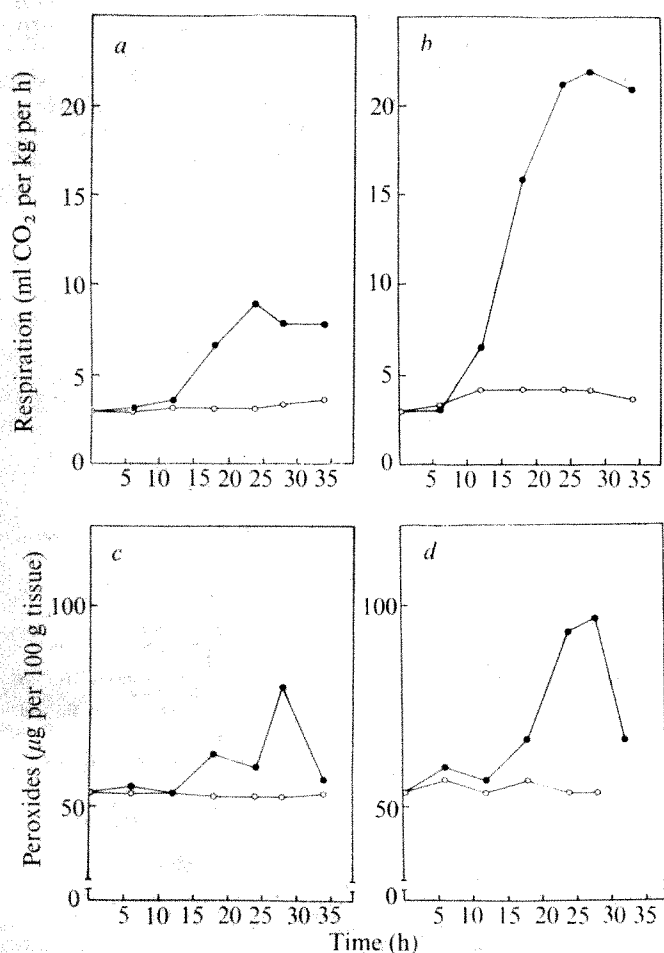
Influence of ethylene and oxygen on respiration and peroxide formation in potato tubers

APPLICATION of ethylene to potato tubers induces a respiratory upsurge¹, comparable with that in ripening climacteric fruit², but which can be studied independently of other ethylene-induced processes occurring in fruit. It has been suggested that ethylene catalyses oxygen-requiring processes, reflecting in part oxygen utilisation in the formation of peroxides³. We report here that high O₂ tensions, in combination with ethylene, markedly enhance the respiratory rise, which is accompanied by a corresponding increase in peroxides.

Locally grown potato tubers (*Solanum tuberosum* L., variety Northchip) were preconditioned at room temperature for 2 weeks after harvest. Four tubers weighing together approximately 1.0 kg were each placed in a 4-l jar and ventilated continuously, at a flow rate of 400 ml min⁻¹, with 21% (air) or 100% O₂, supplemented with 0 or 10 p.p.m. ethylene as previously described³. The potatoes were kept at 21 °C throughout the experiment. Evolution of CO₂⁴ and peroxide⁵ was monitored at 6-h intervals for 36 h. All determinations were run in duplicate.

Figure 1a and b shows that in the absence of ethylene respiration was at a steady rate in both air and 100% O₂, although in oxygen the rate was somewhat higher. Addition of ethylene to air resulted, after a lag, in a respiratory rise, reaching a peak two or three times the initial rate after

Fig. 1 Effect of ethylene on CO₂ evolution (a and b) and peroxide formation (c and d) in air (21% O₂) and 100% O₂. Ethylene concentrations were zero (○) and 10 p.p.m. (●).



24 h, followed by a decline. By comparison, after a comparable lag, ethylene in 100% O₂ induced a rapid rise in respiration to almost ten times the initial rate.

The results show that although ethylene was required to trigger the respiratory rise, as previously observed¹, oxygen tension was the rate limiting factor. A similar interaction between oxygen and ethylene has been obtained in the induction of synthesis of lycopene, the red tomato pigment, in the non-ripening *rin* mutant³. As in potato respiration, ethylene was obligatory for the initiation of lycopene synthesis but O₂ tension was the rate limiting factor.

We hypothesised that the changes in the oxygen tension reflect, in part, peroxide formation and that ethylene triggers the process, because (1) ethylene stimulated peroxide formation in fruit⁶, and (2) peroxide forming enzymes are stimulated by high O₂ tensions⁸.

To test this hypothesis, we measured the changes in peroxides in the tubers as related to the effect of different O₂ tensions with and without ethylene. As with CO₂ evolution in the absence of ethylene, the peroxide level did not change in air or 100% O₂ (Fig. 1c and d, respectively). Addition of ethylene resulted in an upsurge in the formation of peroxides. As with CO₂ evolution, the peroxide levels were stimulated by high O₂ tensions.

Solomos and Laties⁷ proposed that ethylene triggers an alternative respiratory pathway (cyanide insensitive). Our demonstration that the respiratory rise, as influenced by ethylene and oxygen, is accompanied by an upsurge in peroxides suggests that the alternative respiratory pathway leads to the formation of peroxides.

The formation of peroxides in plants is catalysed by several enzyme systems⁸, but may also result from the dismutation of superoxides⁹. In any case, the upsurge in peroxides represents the formation of partially reduced oxygen forms, while in respiration molecular oxygen is reduced completely (formation of water). The action of ethylene, as shown in potatoes, may therefore be to divert the flow of electrons from the complete reduction of molecular oxygen to the formation of partially reduced oxygen forms, such as peroxides, which by comparison with O₂, represent oxygen forms which readily react with cellular constituents^{8,9}. In this way, ethylene may induce redox changes in tissues, including the oxidative breakdown of senescence-retarding hormones in fruits⁹, and thereby the onset of senescence processes.

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Characterisation of human cell lines and differentiation from HeLa by enzyme typing

THE value of a great deal of research on cells in culture depends on the certain identity of the cells under investigation. Contamination of one cell line with another, leading to mixed cultures or in some cases complete overgrowth of the original cells by the contaminating line, is a long-standing problem. Interspecific contamination has been

recognised by both immunological and karyological techniques^{1,2}, but the most striking demonstration of intra-specific contamination was by Gartler³. He presented evidence, based on the detection of common genetically determined variation in two enzymes, that many permanent tumour cell lines, set up originally in several different laboratories, were in fact HeLa cells. Recently the problem of contamination with HeLa has become a focus of general interest and there has been a search for certain chromosomal and other characteristics of HeLa in a large number of established lines⁴⁻⁶. There is no guarantee, however, that the contaminating cell line will always be HeLa and there appears to be a need for a quick and reliable method for the absolute identification of all human cell lines.

In recent years more has become known about genetic diversity in man, and many electrophoretically distinguishable enzyme polymorphisms have been discovered⁷. Most of this information has come from studies on red cells and placenta, but many polymorphic loci are expressed in cultured cells. Thus it is already possible to devise a relatively simple scheme which goes a considerable way towards the positive identification of individual human cell lines. As new polymorphisms are discovered it will doubtless be improved. We present here our current list of enzyme polymorphisms which we have found useful for the identification of all types of cultured cells, including diploid and transformed fibroblasts and permanent lymphoblastoid lines. We have been able to test out the scheme

Table 1 Enzyme loci useful in characterising human cultured cells

Enzyme		Population group*				Refs		
Locus	Phenotype	N. European	Black					
		Frequency of common phenotypes	Chance of 2 people being alike	Frequency of common phenotypes			Chance of 2 people being alike	
<i>PGM</i> ₁	1	0.57		0.62		7		
	2-1	0.37	0.465	0.33	0.496			
	2	0.06		0.05				
<i>PGM</i> ₂	1	1.00		0.98		8		
	2-1	—	1.000	0.02	0.961			
	2	—		<0.01				
<i>PGM</i> ₃	1	0.55		0.14		7+		
	2-1	0.38	0.452	0.47	0.392			
	2	0.07		0.39				
<i>GOT</i> _M	1	0.97		0.85		7+		
	2-1	0.03	0.942	0.02	0.739			
	3-1	—		0.13				
	Others	<0.01		<0.01				
<i>ESD</i>	1	0.82		0.82		7		
	2-1	0.17	0.701	0.17	0.701			
	2	0.01		0.01				
<i>AK</i> ₁	1	0.92		1.00		7		
	2-1	0.08	0.853	—	1.000			
	2	<0.01		—				
<i>ADA</i>	1	0.90		0.94		7		
	2-1	0.10	0.820	0.06	0.887			
	2	<0.01		<0.01				
<i>ACP</i> ₁	A	0.13		0.03		8		
	BA	0.42		0.28				
	B	0.35	0.321	0.69	0.555			
	CA	0.04		<0.01				
	CB	0.06		<0.01				
	Others	<0.01		<0.01				
	<i>PGD</i>	A	0.96		0.88			9
		AC	0.04	0.923	0.11		0.786	
Others		<0.01	<0.01					
<i>G6PD</i>	B	1.00		0.56		10‡		
	A	—	1.000	0.44				
<i>PEP A</i>	1	1.00		0.81		7§		
	2-1	—	1.000	0.18	0.689			
	2	—		0.01				
<i>PEP C</i>	1	0.95		?		11¶		
	4-1	0.05	0.905	?	?			
	Others	<0.01		?				
<i>ACON</i> _s	1	0.98		0.73		7+		
	2-1	<0.01	0.961	0.05	0.575			
	3-1	0.01		—				
	4-1	—		0.20				
	4	—		0.01				
	Others	<0.01		0.01				
Combined chance of two people being alike for all loci			0.025		0.008			

*PGM*₁, *PGM*₂, *PGM*₃: first, second and third loci of phosphoglucosmutase. *GOT*_M: mitochondrial glutamate-oxaloacetate transaminase. *ESD*: esterase D. *AK*₁: adenylate kinase. *ADA*: adenosine deaminase. *ACP*₁: 'red cell' acid phosphatase. *PGD*: phosphogluconate dehydrogenase. *G6PD*: glucose-6-phosphate dehydrogenase. *PEP A* and *PEP C*: peptidases A and C. *ACON*_s: soluble aconitase. Enzymes bracketed together were routinely run on the same gel. Electrophoresis and detection of enzymes were by methods previously described⁷.

*The phenotype frequencies of many of the enzymes show marked regional variations even within the same ethnic group. The most reliable data are from the North European population surveys. Wherever possible the phenotype frequencies given for the Black population are derived from population studies on Yorubas from Nigeria.

†Enzyme not found in red cells.

‡The *G6PD* A phenotype includes both A(-) and A(+), since these are difficult to distinguish in cultured cells. The frequency of the A and B phenotypes is very variable in different populations and this estimate is from Nigerian males. We have used the male frequencies to calculate the chance of two cell lines being alike since a line in long-term culture might be expected to be effectively monoclonal and thus to express only 1 allele at an X-linked locus such as *G6PD*.

§The frequencies given for *PEP A* ignore the *PEP A*⁺ allele which is technically difficult to distinguish from *PEP A*¹.

¶Some alleles at the *PEP C* locus are not expressed in red cells and reliable population data are not available for the Black population (see refs 7 and 11).

on five permanent epithelial cell lines derived from human bladder tumours. These results are presented together with findings on nine other permanent human cell lines, all of which seem to be, or are known to be, HeLa.

Details of the enzymes used in our cell identification scheme are given in Table 1. The loci selected are in general those which have useful gene frequencies in different populations, and which have been studied in detail in both cultured cells and in families. All the loci are expressed in other tissues, and ten of them are expressed in red cells (Table 1) so that the best positive check on the identity of a cell line can be made if blood or other tissue is set aside for enzyme testing when the cells are put into culture. Using starch gel electrophoresis it is not necessary to run a separate gel for each enzyme. As Table 1 shows, the seven enzymes providing the most information (the first seven tested, excluding PGM₂) are usually run on only three gels, and could be done on fewer if necessary. Using routine methods the enzymes suggested could all be done on a single pellet of 10⁷ cells.

Three of the enzymes listed in Table 1 require further comment. Adenosine deaminase (ADA) and occasionally acid phosphatase (ACP₁) may be difficult to type in diploid fibroblasts, because of a preponderance of 'tissue' isozymes in which genetic variation is not detectable. The adenylate kinase locus (AK₁) is not expressed in lymphoid lines. In our experience all the loci listed in Table 1 are readily typeable in permanent epithelial lines.

Table 1 also shows the chance of being able to distinguish any two individuals, of similar racial origin, using these enzyme loci; this turns out to be about 97% in North Europeans and about 99% in Blacks. The chance of distinguishing any two lines of dissimilar racial origin would of course be greater. Further information may be obtained by testing other enzymes which have been omitted from this scheme because variants occur only at low frequencies. Some cell lines would be found to be heterozygous for one of these variants if sufficient loci were tested¹². There are also several fairly recently discovered enzyme polymorphisms that have not yet become established in our laboratory as routine markers for cultured cells, but which will probably be useful in the future. These include mitochondrial malic enzyme^{13,14}, glyoxalase I¹⁵ and uridine monophosphate kinase¹⁶. α -Fucosidase may also be included in the scheme, but it requires an additional technique, isoelectric focusing^{17,18}.

The results of testing a series of human cell lines by the scheme outlined above are given in Table 2. Five lines were derived from bladder tumours and maintained at the

Imperial Cancer Research Fund Laboratories. All these were of North European origin: RT4 was an English male¹⁹; RT112 an English female (C. C. Rigby and L.M.F., unpublished observations); T24 was a Swedish female²⁰; J82 was a Swedish male (provided by Carol O'Toole, University of California) and EJ was an American male (provided by J. Daly, Massachusetts General Hospital). Most of the other lines are well known strains of HeLa and all except BU25 (ref. 21) have featured in a recent investigation of cell identity⁵. The two remaining lines, LU and EUE, were originally set up as independent lines from foetal lung and subcutaneous foetal tissue respectively^{22,23}. They were sent to us for typing because they were suspected of having been taken over by HeLa and in fact the enzyme types were found to be those characteristic of HeLa cell lines. All the cells tested were sent to the Galton Laboratory in the form of washed cell pellets.

Clearly the bladder tumour lines are not the same as HeLa and differ among themselves. Three of them have unique enzyme phenotypes. The other two, J82 and EJ, are the same as each other on the enzymes tested in Table 1 and so appear to be identical. This is unfortunate but statistically not too distressing since if the chance of any two individuals being the same is 3%, the chance of finding two alike in a sample of five lines is about 1 in 4. Thus the evidence at present is not strong enough to suggest that one line has been taken over by the other, but clearly this is a possibility. Attempts to distinguish the two lines using other enzymes including α -fucosidase and glyoxalase I have so far been unsuccessful. They both seem to be phenotype α FUC 2-1 and GLO 1. The chance of two lines from different individuals being identical for these two enzymes is about 1 in 4. The two lines do, however, differ in other properties (C. J. Marshall, L.M.F. and A. W. Carbonell, in preparation). EJ cells produce tumours in nude mice and have a colony-forming efficiency in agar of 23%. J82 cells do not form tumours in nude mice in our experiments and have a colony-forming efficiency in agar of 3%. The problem of identification encountered here illustrates the usefulness of directly testing material such as red cells, white cells, or in these cases samples of the tumours from the original donors.

The reliability of enzymes as genetic markers for cell identification depends on the stability of the enzyme phenotypes in culture. We have attempted to check this particular point by repeatedly testing cells in culture over various periods of time. Whenever possible, material from the original donor has also been examined. We have monitored more than 100 lymphoid lines from more than

Table 2 Enzyme phenotypes in five bladder tumour lines and some different strains of HeLa

Enzyme	Bladder tumours					HeLa	HeLa _{s3}	HEP-2	D98	FLA	BU25	KB	LU	EUE
	T24	RT4	J82	RT112	EJ									
PGM ₁	1	2-1	1	1	1	1	1	1	1	1	1	1	1	1
PGM ₂	1	1	1	1	1	1	1	1	1	1	1	1	1	1
PGM ₃	1	2-1	1	2-1	1	1	1	1	1	1	1	1	1	1
GOT _M	1	1	1	2-1	1	1	1	1	1	1	1	1	1	1
ESD	1	2-1	1	2-1	1	1	NT	1	1	NT	NT	NT	NT	NT
AK ₁	1	1	1	1	1	NT	NT	NT	1	NT	1	1	1	1
ADA	1	1	1	1	1	1	1	NT	1	NT	1	1	1	1
ACP ₁	BA	A	B	B	B	NT	BA	NT	BA	NT	BA	BA	NT	NT
PGD	A	A	A	A	A	A	A	NT	A	A	A	A	A	A
G6PD	B	B	B	B	B	A	A	A	A	A	A	A	A	A
PEPA	1	1	1	1	1	1	1	1	1	NT	1	1	1	1
PEPC	1	1	1	1	1	1	1	1	1	NT	1	1	1	1
ACON _s	NT	NT	NT	NT	NT	1	NT	NT	1	NT	NT	NT	NT	NT

In addition to the enzymes shown here D98 was tested for lactate dehydrogenase (LDH_A and LDH_B), mannose phosphate isomerase (MPI), superoxide dismutase (SOD_A), soluble and mitochondrial malate dehydrogenase (MDH_s and MDH_M), isocitrate dehydrogenase (ICD_s), purine nucleoside phosphorylase (NP), glucose-phosphate isomerase (GPI), adenine phosphoribosyl transferase (APRT), enolase (ENO₁), glyoxalase I (GLO) and mitochondrial malic enzyme (ME_M). It seemed to have the common homozygous phenotype at all these loci with the exception of ME_M, for which no satisfactory result was obtained. However, two other HeLa strains tested for ME_M (HELA_{s3} and LU) were clearly heterozygotes, phenotype ME_M 2-1.

70 people, about 100 primary fibroblast cultures and a small number of permanent epithelial lines in addition to those described here. From one lymphoid line more than 500 clones were examined, each for an average of 30 loci, 300 of these clones having been treated with large doses of mutagen (ref. 24 and further unpublished data). The total number of tests performed has been more than 20,000, since many lines have been checked repeatedly. In all these cells only two examples of a true mutation, involving alteration of electrophoretic mobility, have been seen (ref. 23 and unpublished work). Loss of enzyme activity, often involving only one allele so that an apparent homozygote is produced, has also been observed but with very low frequency²⁴. In several of these cases this was associated with a specific chromosomal aberration²⁵. On 15 other occasions an apparent change in phenotype was observed, three times in fibroblast cultures and 12 times in lymphoid lines. These changes were conclusively shown to be due to contamination by checking other enzymes and by karyotype analysis.

Clearly more extensive investigations into the identity of cell lines would be desirable, and other workers have suggested HLA typing²⁶ and monitoring of the karyotype, particularly for the presence or absence of a Y chromosome⁶. Both of these methods have many advantages which we do not wish to ignore. We feel, however, that the routine testing of enzyme phenotypes, while perhaps not yet equalling the theoretical discriminating power of the HLA system, is such a reliable and simple tool for the characterisation of human cultured cells that it should be adopted with much greater enthusiasm than hitherto.

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Phenotypic reversion of ricin-resistant hamster fibroblasts to a sensitive state after coating with glycolipid receptors

THE cytotoxicity of the plant lectin ricin, of *Ricinus communis* seeds, towards mammalian cells is mediated in three steps¹. Binding of the toxin to cell surface galactose or *N*-acetylgalactosamine receptors is followed by entry of the bound lectin into the cells, perhaps by endocytosis^{2,3} and inhibition of protein synthesis⁴⁻⁶. Several variant lines of cells selected for resistance to ricin have been shown⁷⁻¹⁰ to bind ricin poorly and to carry much smaller amounts of receptors at the cell surface. We report here that when such a resistant cell line of baby hamster kidney (BHK) fibroblasts^{8,9} is incubated with a ricin-binding glycolipid fraction prepared from human erythrocytes, glycolipid is taken up and the cells exhibit increased binding of ricin and greater sensitivity to lectin-mediated inhibition of cell protein synthesis. These studies suggest that glycolipids can function in certain circumstances as membrane receptors in the binding of ricin and mediation of ricin cytotoxicity.

Baby hamster kidney (BHK) cells and the ricin-resistant variant, Ric^R21 were grown at 39 °C in Glasgow modified Eagle's medium (GMEM) supplemented with 10% tryptose phosphate and 10% foetal bovine serum as described previously^{8,9}.

A ricin-binding glycolipid fraction was isolated from human blood group O erythrocytes as described before¹¹⁻¹³. The fraction soluble in chloroform:methanol:water (1:2:1) containing fucose (8%), galactose (41%), glucosamine (33%), fatty acids (4%) and sphingosine (5%) was evaporated to dryness just before use, dissolved in water at 5 mg per ml and the resulting transparent aqueous solution was sterilised by filtration. ¹⁴C-labelled glycolipid was prepared by de-*N*-acetylation in *N*-sodium hydroxide at 100 °C for 30 min and reacylation with ¹⁴C-acetic anhydride after neutralisation and dialysis. The specific activity was 1.3×10^6 c.p.m. mg⁻¹.

As Fig. 1 shows, when cells were incubated in phosphate-buffered saline (PBS) with ¹⁴C-labelled glycolipid about 2% of the labelled glycolipid was taken up in cell-bound form regardless of the initial concentration of glycolipid added. Maximum uptake by cells required 4 h of incubation with the glycolipid fraction at 39 °C. Measurable binding did not occur at 2 °C. Cells incubated for 4 h at 39 °C with glycolipid ($50 \mu\text{g ml}^{-1}$) incorporated, in several independent experiments, $5-12 \times 10^6$ molecules per cell. At this time no radioactivity was released from the cells by brief trypsinisation ($2 \mu\text{g ml}^{-1}$ at 39 °C for 5-15 min) indicating that the cell-bound glycolipids were not loosely attached, for example, to serum proteins adsorbed at the cell surface.

Incorporated glycolipid increased the binding of radioiodinated ricin to normal BHK cells as well as to the ricin-resistant variant, Ric^R21 (Fig. 2). With cells containing 5×10^6 molecules of glycolipid per cell, ricin binding was enhanced to a similar extent by approximately 3×10^6 sites per cell. The enhancement was most marked with Ric^R21 cells which by themselves show only about 10% of the ricin-binding capacity of normal BHK cells (Fig. 2). After glycolipid treatment ($50 \mu\text{g ml}^{-1}$) ricin binding was increased threefold. The enhancement of ricin binding was

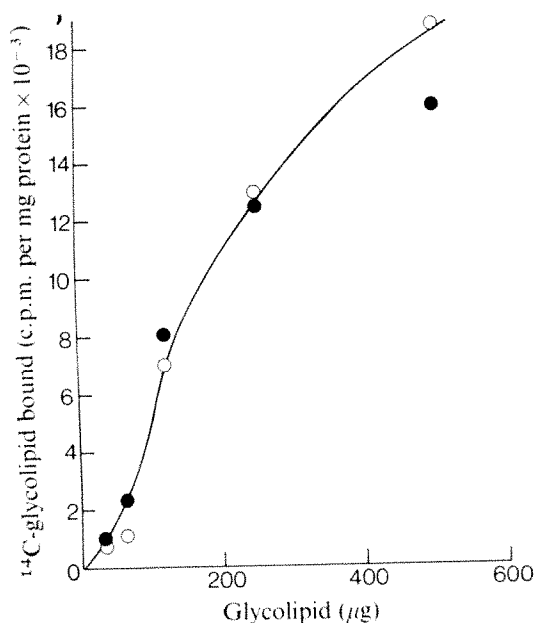


Fig. 1 Binding of ^{14}C -labelled human erythrocyte glycolipids to BHK cells. Confluent monolayers of parental BHK cells (●) or ricin-resistant variant (○), Ric^R21, on 35-mm diameter plastic dishes (4×10^6 cells per dish) were exposed in 1 ml of PBS to various concentrations of the ^{14}C -labelled glycolipid fraction at 39 °C for 4 h. The saline was removed, the cells were washed three times with saline and then solubilised in 1 ml of N-NaOH and counted for bound radioactivity. Other portions were assayed for protein.

greater, about sixfold, after treatment of Ric^R21 cells with glycolipid at $500 \mu\text{g ml}^{-1}$, and ricin binding then approximated to that of normal BHK cells. Ricin binding to normal BHK cells was doubled after similar treatment. But the enhancement of ricin binding, $6-7 \times 10^6$ sites per cell, was considerably less than the numbers of glycolipid molecules bound to the cells after treatment with $500 \mu\text{g}$ of glycolipid. We conclude that cells exposed to very high concentrations of glycolipids ($500 \mu\text{g ml}^{-1}$) take up glycolipids either intracellularly or in surface sites that are not accessible to ricin added externally. Perhaps in these conditions glycolipids in part were sequestered intracellularly by pinocytosis. We do know, however, that the glycolipids used in this work can be incorporated into erythrocytes in which pinocytosis is improbable, and confer Lewis and ABO-blood group activity on these cells¹³.

Whatever the mechanism of incorporation¹³, treatment of BHK cells with glycolipids, in conditions in which an association with the cell surface could be demonstrated by an enhancement of ricin binding, was found to increase the sensitivity of the cells to ricin. Ricin-mediated inhibition of cell protein synthesis was measured as follows. Confluent monolayers of normal BHK cells or ricin resistant Ric^R21 variant cells growing on 35-mm diameter plastic dishes were incubated in GMEM minus serum (1 ml) with ricin ($1 \mu\text{g}$ or $10 \mu\text{g}$). After 1 h at 39 °C, 1 ml of ^3H -leucine ($3.3 \mu\text{Ci}$, 59 Ci mmol^{-1}) in leucine-free GMEM was added to each dish. Incorporation was carried out over 1-6 h when the medium was removed and the cell monolayers were washed three times with PBS, twice with cold (2 °C) 2% phosphotungstic acid-10% perchloric acid and twice with cold alcohol. The washed monolayers were then dissolved in hot (80 °C) N-NaOH

Table 1 Inhibition of BHK cell protein synthesis by ricin

Experiment	Cells	Glycolipids ($\mu\text{g ml}^{-1}$)	Ricin ($\mu\text{g ml}^{-1}$)	^3H -leucine incorporation (c.p.m. per mg protein)	Incorporation (% control)		
1	Normal BHK	None	0	64,000	100		
			1	13,400	21		
			10	7,050	11		
		50	0	69,000	100		
			1	6,950	10		
			10	7,510	12		
	500	0	53,360	100			
		1	5,810	11			
		10	2,660	5			
		Ric ^R 21	None	0	77,500	100	
				1	78,350	196	
				10	75,800	98	
	50		0	78,300	100		
			1	76,000	97		
			10	37,600	48		
	500	0	73,500	100			
1		49,300	67				
10		17,400	23				
2		Ric ^R 21	None	10	212,000	100	
				10	75,500	37	
			250	10	74,000	33	
	500		10	47,000	22		
	500		10*	172,000	86		
	500		10	170,000	100		
	3		Ric ^R 21	None	10	127,000	74
					10	68,000	40
200		10		153,000	90		
50†		10		156,000	92		
200†		10					

Cells were incubated for 4 h at 39 °C with or without erythrocyte glycolipid fraction as indicated. The cells were then washed and incubated with ricin ($1 \mu\text{g ml}^{-1}$ or $10 \mu\text{g ml}^{-1}$) for 1 h at 39 °C before assay of ^3H -leucine incorporation into cellular protein. Control dishes contained no ricin and showed that treatment of cells with glycolipid had no effect on the level of ^3H -leucine incorporation into protein.

*This solution was supplemented with 10 mM lactose.

†A degraded glycolipid derivative which shows no ricin-binding activity was prepared¹³ by Smith oxidation with 40 mM sodium periodate in 50 mM sodium acetate buffer, pH 4.5 overnight at 4 °C to destroy galactose terminal units. Excess oxidant was destroyed with ethylene glycol and dialdehydes were reduced with sodium borohydride at room temperature for 1 h. Excess borohydride was destroyed by acetic acid and acetal linkages were hydrolysed in 0.5 N HCl at 20 °C for 8 h. Finally the degraded glycolipid fraction was dialysed extensively against water and freeze dried. The material was then dissolved (5 mg ml^{-1}) in water.

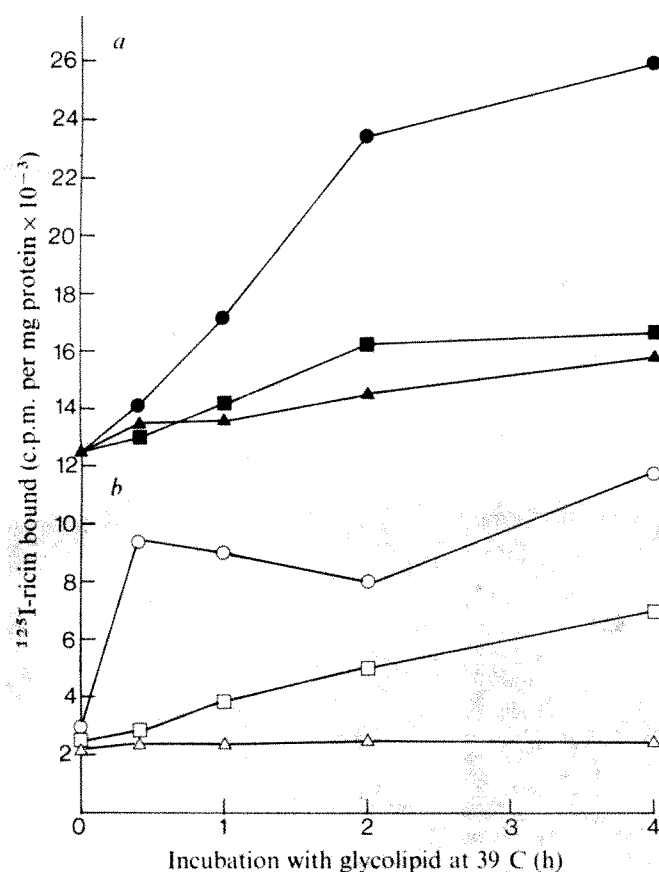


Fig. 2 Binding of ^{125}I -ricin to BHK cells before and after treatment with erythrocyte glycolipids. Normal cells (a) and ricin-resistant variant Ric^{R21} (b) were grown to confluency on 35-mm diameter plastic dishes (4×10^6 cells per dish). Growth medium was removed and duplicate monolayer cultures were incubated at 39 °C with 50 µg or 500 µg of glycolipid in 1 ml of PBS. Control dishes were incubated with PBS. At various times, unbound glycolipid was removed by washing three times with saline and ^{125}I -ricin (50 µg, 3.5×10^5 c.p.m.) in PBS (1 ml) added at 2 °C. After incubation of control cells and glycolipid-treated cells with ricin for 60 min at 2 °C the monolayers were washed three times with saline, dissolved in 1 ml N-NaOH and counted. Ricin binding is expressed in terms of cellular protein and corrected for non-specific binding of ^{125}I -ricin to cells (control or glycolipid-treated) in the presence of hapten inhibitor (10 mM lactose) and to dishes containing no cells to which appropriate amounts of glycolipid had been added, incubated at 39 °C for the stated times and then washed with saline. In no case did the nonspecific binding (lactose irreversible counts or counts bound to dishes) exceed 20% of the radioactivity bound in the absence of lactose to cell layers in identical conditions. ● and ○, 500 µg glycolipids; ■ and □, 50 µg glycolipids; ▲ and △, controls.

(1 ml) and portions (0.2 ml) were removed for radioactive counting and protein assays. Incorporation of ^3H -leucine into protein by normal BHK cells was inhibited about 80%, by ricin at $1 \mu\text{g ml}^{-1}$ and 90% by ricin at $10 \mu\text{g ml}^{-1}$ (Table 1, experiment 1). In contrast, protein synthesis of the resistant variant, Ric^{R21}, was unaffected by these concentrations of ricin (Table 1, experiment 1). As Table 1 shows, in Ric^{R21} cells incubated with glycolipids for 4 h at 39 °C and then, after washing, with ricin ($1 \mu\text{g}$ or $10 \mu\text{g}$) for 1 h, the incorporation of ^3H -leucine into protein was decreased significantly compared with cells not pretreated with glycolipids. The already high sensitivity of normal BHK cells to these concentrations of ricin also appeared to be significantly increased by pretreating cells with glycolipids (Table 1, experiment 1). The magnitude of the effect of Ric^{R21} cells was dependent on glycolipid concentration to some extent (Table 1, experiment 2). Furthermore, the effect of ricin on Ric^{R21} cells pretreated with glycolipids was abolished by inclusion

of 10 mM lactose in the culture medium (Table 1, experiment 2). Additional evidence that the potentiation of ricin cytotoxicity by pretreatment of Ric^{R21} cells with glycolipids requires binding of lectin to the carbohydrate moiety of the glycolipids was obtained using a degraded glycolipid fraction (Table 1, experiment 3). Oxidation of the glycolipid fraction with periodate destroys preferentially the ricin-binding terminal galactose residues of the molecule¹³. When this material was incorporated into Ric^{R21} cells, there was no change in the high resistance of the cells to ricin (Table 1, experiment 3).

We conclude from our results that a ricin-binding glycolipid fraction extracted from human erythrocytes when added to baby hamster kidney fibroblasts becomes tightly associated with the cells. Such incorporation increases the numbers of toxin molecules that can bind to the cells and the cells concurrently become considerably more sensitive to the cytotoxic action of ricin as indicated by a decreased incorporation of ^3H -leucine into cellular proteins. It is unlikely that complex glycolipids containing twenty or more monosaccharides and conferring blood group specificity on human erythrocytes exist endogenously in hamster fibroblasts. But other ricin-binding glycolipids, for example ganglioside GM1 (ref. 14), are known that could function as the normal receptors mediating ricin toxicity in sensitive BHK cells. Preliminary evidence shows no detectable levels of GM1 or GM2 in either normal BHK cells or Ric^{R21} cells, however, and the levels of other galactolipids, GM3 (hematoside) and lactosylceramide, are similar in the two cell lines. We therefore favour the alternative interpretation that incorporation of relatively large amounts of "foreign" glycolipids into the BHK surface membrane can mimic the normal route, for example through glycoprotein receptors, by which the toxin enters these cells.

It is clear that the incorporated glycolipids are less efficient in mediating ricin toxicity than are the natural receptors. Thus, Ric^{R21} cells that bound sufficient glycolipid to enhance ricin binding to approximately the level exhibited by normal BHK cells (6×10^6 sites per cell) were only about half as sensitive to ricin (Table 1, experiment 1). The mechanism by which glycolipid incorporated into the surface membrane participates in ricin cytotoxicity is, of course, unknown. It may do so directly, or by association with integral glycoproteins, perhaps those that in normal cells carry the ricin-binding sites. We have no unequivocal way of deciding between these two mechanisms, neither of which would be expected to be as efficient as the normal process. In the latter case, it may be that the association of glycolipid with putative glycoprotein mediators of ricin toxicity involves only a small proportion of the total glycolipid incorporated into the cell membrane. Perhaps only when the membrane is heavily loaded with glycolipid does the number of glycoprotein molecules "primed" with glycolipid receptors reach a threshold value necessary for permeation of ricin into the cells.

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Cellular adhesiveness reduced in ricin-resistant hamster fibroblasts

THE means by which tissue cells adhere to one another and to non-cellular surfaces is not known, although complex carbohydrate of their surface membrane may be involved^{1,2}. To test this, one can ask whether mutations which affect

the structure of surface carbohydrate also alter cellular adhesiveness. Meager *et al.*³ have isolated a series of stable variants of baby hamster kidney fibroblasts (BHK 21, clone 13) which are resistant to the toxic lectin ricin. The first step in killing of cells by ricin is binding of the lectin to galactosyl- or *N*-acetylgalactosaminyl residues at the cell surface^{4,5}, and many ricin-resistant clones yield cell lines with reduced glycosylation of their cell surfaces³. We now report that, judged by their ability to form aggregates in suspension, the intercellular adhesiveness of a number of ricin-resistant lines is less than unselected lines. We have also found that cells of most lines attach less readily than controls to glass coverslips bearing adsorbed films of serum or gelatin.

After 24 h of incubation in a gyratory shaker⁶, suspensions of control cells yield compact spherical aggregates approximately 200 μ m in diameter (Fig. 1). Ricin-resistant

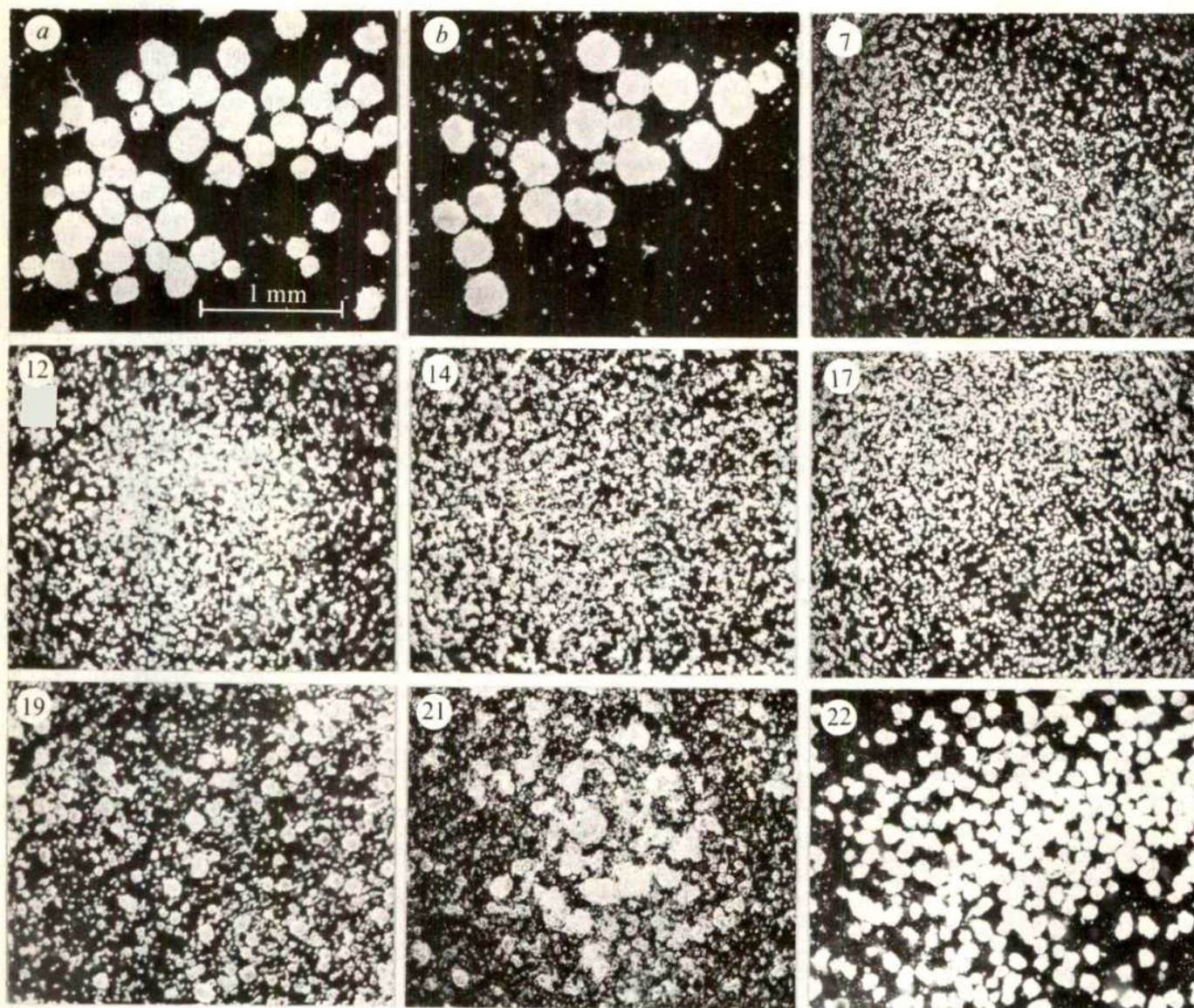


Fig. 1 Darkground micrographs of aggregated suspensions of BHK 21 cells and ricin-resistant derivatives after gyratory shaking for 24 h. *a*, C13 cells from which ricin-resistant cells were selected. *b*, Mutagenised but unselected control (see below) and resistant lines as numbered. Confluent cultures grown in Glasgow-modified Eagle's medium supplemented with 10% foetal bovine serum and 10% tryptose phosphate (EFT) were dispersed using EDTA and trypsin¹⁷. Cells were resuspended in EFT at 5.10^6 per ml. 2 ml of each suspension was cultured in a 25-ml silicone-treated conical flask, with gas phase air, 95%, CO_2 5%, in a gyratory shaker⁶ at 70 r.p.m. 37 °C for 24 h. 0.25-ml samples were photographed in a dish, diameter 14 mm. C13 cells from which ricin-resistant cells were selected at Mill Hill (*a*) in Figs 1-3) showed adhesive properties very similar to Glasgow stocks used in earlier experiments^{8,17}. Two further control lines were obtained by mutagenising C13 cells as for selection, but then growing out the subclones in the absence of ricin. Our unpublished experiments show that cells of lines Ric¹⁴, 19 and 21, but not 22, have lost the ability to readhere in the early phase of aggregation which occurs in the first hour of shaking of freshly suspended cells¹⁷. This assay is not satisfactory for comparing lines selected from C13 cells, because it is relatively easy to obtain from C13 stocks without selection, lines which do not aggregate in the first hour from trypsin dispersal¹⁸. Lines of this phenotype do however aggregate very similarly to unrecycled C13 cells when cultured for 24 h in a gyratory shaker.

(Ric^R) lines exhibit a range of reduced aggregation behaviour, from Ric^R22 which differs from controls only in forming smaller aggregates, to Ric^R7 and 17 which aggregate very little. Quantitation by use of a Coulter counter shows that these comparisons are repeatable and stable through a month's consecutive passage of the cells (Fig. 2) and after storage of cells in liquid nitrogen for 1 yr. Figure 3a shows that freshly dispersed cells of Ric^R19 and 22 adhere to a film of adsorbed foetal bovine serum to the same extent as controls, whereas the remaining six tested adhere less well. This behaviour is not specific to foetal calf serum, for the pattern is similar with calf serum and gelatin (Fig. 3b), although there is much more day to day variation on these latter films.

Ric^R22 cells exhibit at confluence the parallel alignment characteristic of the parental cells, bind normal levels of ricin^{3,7} and as shown here, also resemble wild type in adhesive behaviour. This agrees well with the evidence that the moderate resistance to ricin of this line depends on a cytoplasmic, rather than a surface alteration⁷. Ric^R12, 16 and 19 are lines which do not show a reduction in ricin binding, but nevertheless may have reduced surface glycosylation, since they are cross resistant to the lectin from *Phaseolus vulgaris*³. The remaining lines, including those

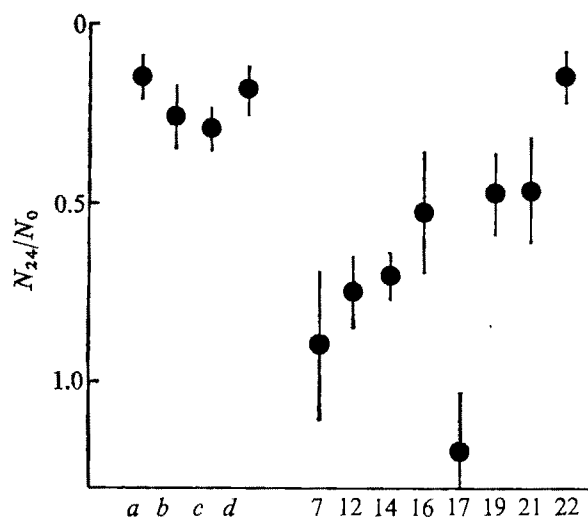


Fig. 2 Aggregation of suspensions of BHK 21 cells and ricin-resistant derivatives measured using an electronic particle counter. *a*, C13 cells from which ricin-resistant cells were selected; *b* and *c*, mutagenised but unselected lines; *d*, Glasgow stocks of C13 cells. 0.1-ml samples of suspensions aggregated as for Fig. 1 were diluted in 20 ml of 0.9% w/v sodium chloride and counted in a Coulter counter Z_B, using a 200-μm aperture⁴. Data are the concentration at 24 h of particles of all sizes divided by the value before incubation. For each cell line, means and standard deviations are shown from six to eight separate experiments, spread through about eight consecutive passages of the cells. The count does not reflect the tendency of Ric^R22 to form smaller aggregates than wild-type cells since the assay is dominated by the proportion of particles representing single cells and very small clusters. Values greater than 1 for Ric^R17 indicate limited cell division in the cultures.

most conspicuously affected in aggregation (7 and 17) all bind only 10–40% as much ricin as do wild type³. The aggregation properties of these lines are therefore broadly consistent with the proposal that intercellular adhesion requires carbohydrate chains which are potential acceptors of terminal sialyl residues^{8–10}. Indeed this 'sialyl acceptor' model predicts a strong correlation between ricin resistance and decreased intercellular adhesiveness, for those monosaccharide residues often terminal in sialic-acid free carbohydrate chains (galactose or *N*-acetylgalactosamine), are the same as those required for ricin binding. Besides these residues, the model requires the existence of a cell-surface protein with carbohydrate-binding activity (perhaps a sialyl transferase³ or lectin-like protein¹¹) of specificity resembling

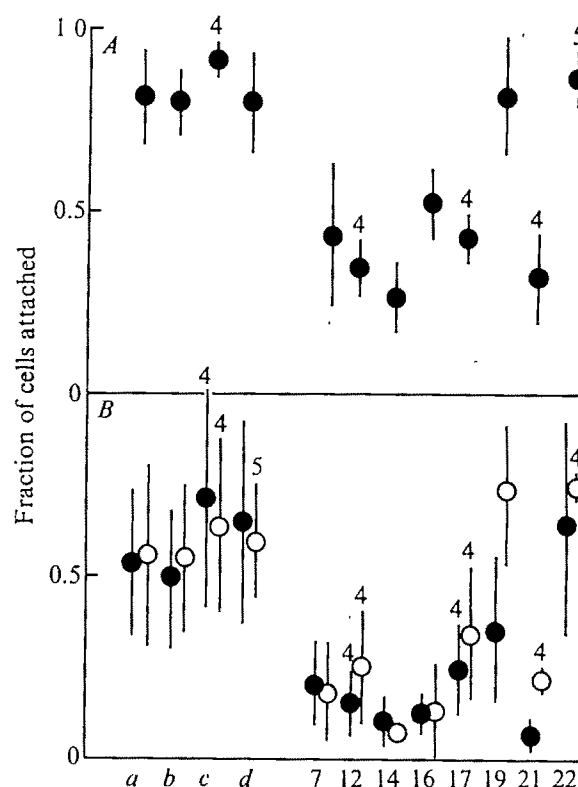


Fig. 3 Attachment of BHK 21 cells and ricin-resistant derivatives to glass coverslips bearing adsorbed protein films. *A*, foetal bovine serum; *B*, calf serum (solid symbols) gelatin (open symbols). *a–d* as Fig. 2. Data are the fraction of cells which had attached after 45 min. Mean and standard deviation of 4–14 experiments on each line, number shown where less than six. Attachment was measured much as described by Rabinovitch and De Stefano¹⁹ except that cells were labelled by incorporation of ³²P-phosphate. Cells were grown for 24 h in medium minus tryptose phosphate (EF) containing 2 μCi of ³²P-phosphate per ml, which was then replaced with unlabelled EFT for a further 18 h growth. Cells dispersed with EDTA and trypsin were re-suspended in Hanks medium, pH 7.2, buffered with 0.01 M HEPES medium at 10⁶ cells per ml. Protein-coated coverslips¹⁸, diameter 13 mm, were drained but not allowed to dry, immediately before receiving 0.1 ml of cell suspension. After incubation for 45 min in a humid atmosphere at 37°C, the coverslips were rinsed in Hanks-HEPES, drained and transferred directly to scintillation phials for counting without further processing. Data are c.p.m. retained as a fraction of total c.p.m. in 0.1 ml cell of suspension, from triplicate coverslips in each experiment.

ricin. We have found that homogenates of BHK cells contain a haemagglutinin selectively inhibited by *N*-acetylgalactosamine and D-fucose¹², but we do not yet know if it is involved in the adhesive phenomena described here. The sialyl-acceptor model does not readily explain the simultaneous effect of the mutations in most lines on both intercellular adhesion and adhesion to substratum. Possibly carbohydrate binding is involved in some purely *cis* interaction (such as clustering in the plane of the surface of a glycoprotein involved in adhesion) rather than, or in addition to, a *trans* role (Fig. 4). Alternatively, one of the interacting components could simply adsorb to the substratum¹². We should stress, however, that there is no direct evidence that protein-carbohydrate 'recognition' is involved in any adhesive properties of these cells. Indeed interpretation of the adhesive changes as a direct result of decreased cell-surface glycosylation may yet prove to be an oversimplification to, a *trans* role (Fig. 4). Alternatively, one of the inter-resistant BHK cell lines lacks a glycoprotein of approximate molecular weight 250,000 which can be labelled by lactoperoxidase-catalysed iodination¹⁴, and such surface components may themselves be involved in cell adhesion^{13,15}.

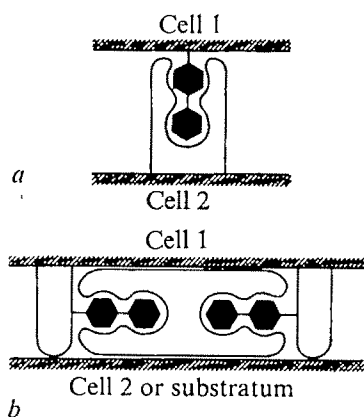


Fig. 4 Possible roles for carbohydrate-protein binding in cell adhesion. *a*, Sialyl-acceptor model⁸, in which an oligosaccharide (solid hexagons) of cell-surface carbohydrate, with terminal galactose or *N*-acetylgalactosamine is bound by a specific receptor on a second cell. *b*, Alternative interaction in which a similar receptor system is involved in aggregation of a glycoprotein at regions of cell-cell or cell-substratum contact.

In lines such as Ric³14 which show lowered adhesiveness, and yet seem to contain normal levels of this component, the glycoprotein could bear a structural alteration affecting its function in adhesion, but not its presence at the cell surface.

Finally, although many ricin-resistant BHK cell lines resemble in adhesive properties certain lines of virus transformed cells¹⁶, none of the lines used in these experiments exhibits a transformed phenotype with respect to growth, since they grow poorly in low serum and in agar suspension, and yield saturation densities similar to the parental line.

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Haemopoietic stem cells are organised for use on the basis of their generation-age

THE spleen-colony assay of Till and McCulloch¹, which is a clonal assay², measures the content of haemopoietic cells in blood-forming tissues. Stem cells (colony forming units in spleen, CFUS) form red cells, granulocytes, platelets and themselves (self-renewal) in these colonies^{3,4}. Worton *et al.*⁵ reported that stem cells separated by velocity sedimentation differed in their capacity for self-renewal. Schofield and Lajtha⁶ reported that CFUS from mice treated with isopropyl methane sulphonate have a low self-renewal capacity. We have studied the functional capacity of myeloid stem cells from normal mice (NBM) and mice treated with hydroxyurea. CFUS from mice treated with five injections of hydroxyurea (5 HUBM, G.S.H. and N. M. Blackett, unpublished) formed 3 times more CFUS and 2.5 times more colony forming units in culture per original colony (CFUC, a committed precursor of granulopoiesis⁷) than those from NBM. When these grafted CFUS were re-transplanted they still formed three times more CFUC and CFUS per secondary colony than those from NBM (Tables 1 and 2).

Table 1 Cellular products of the first graft of NBM and 5HUBM

Group	Colonies per spleen ±s.e.m.	CFUC per spleen ±s.e.m. (×10 ⁻⁶)	CFUC per colony ±s.e.m. (×10 ⁻³)	CFUS per original colony
IC	0.3±0.3	0		
NBM	34.8±1.3	1.46±0.34	4.23±1.0	43.2±2.3
5HUBM	51.3±1.9	5.53±0.61	10.84±1.26	128.2±8.3

NBM donors were 10 normal 3-month BALB/c female mice. 20 similar mice received hydroxyurea (Calbiochem) intraperitoneally (1 mg per g body weight) -32, -26, -10, -7 and -2 h before collection⁸. These were 5HUBM donors. Recipients were 2.5-3-month-old male and female BALB/c (20 mice per group). They were irradiated before grafting (700 R, 230 kVp (kilovolt peak)), HVL (half value layer) 0.5 mm Cu, 146 R.min., TSD (target-source distance) 50 cm., Maximar). Ten days after grafting half the recipients in each group were killed. Their spleens were fixed in formalin-acetic acid-ethanol (1:0.5:8.5) and the surface colonies counted. Twelve days after grafting the remaining recipients were killed. Their spleens were collected aseptically, passed through sterile stainless steel sieves and gauze and assayed individually for CFUC and in pooled groups for CFUS (20 recipients per group). CFUC were cultured in sealed, humidified boxes and counted on day 14. Control dishes with NBM (and the same source of colony-stimulating factor) formed 400 colonies per 10⁴ nucleated cells.

An hypothesis to explain these differences proposes that the use of haemopoietic stem cells to satisfy haemopoietic stress is based on the generation-age of these cells. A young stem cell is one that has undergone few generations since its origin, consequently its capacity for self-renewal and the production of committed precursors is greater than that of an older stem cell which has undergone more generations. Consider the differences between 5HUBM and NBM in the light of this proposal. The effect of the first two doses of hydroxyurea is to strip the haemopoietic tissues of dividing progenitors⁹ and, consequently, of their products. As a result CFUS move into cycle and are, in their turn, killed by further injections of hydroxyurea. It is assumed that the normal regulatory mechanisms bring the more mature CFUS from the pool of CFUS into cycle. On d 2 of treatment with hydroxyurea those cells in the S phase of the cell cycle are killed and the rest gathered at the G₁-S border by injections at -10 and -7 h. As the drug is cleared the blocked cells move into S and are killed by the fifth dose of hydroxyurea at -2 h. Those cells which survive the fifth dose of hydroxyurea are functionally more efficient than those which have survived four injections. (Spleenic DNA and blood ⁵⁹Fe uptake are more than 1.5 times greater and

Table 2 Cellular products of retransplanted stem cells from NBM and 5HUBM

Group	Cell dose in splenic equivalents of the primary graft	Colonies per spleen \pm s.e.m.	CFUC per spleen \pm s.e.m. ($\times 10^{-3}$)	CFUC per secondary colony \pm s.e.m. ($\times 10^{-3}$)	CFUS per secondary colony \pm s.e.m.
IC	0.03	3.9 \pm 1.3	0.01 \pm 0		
NBM	0.03	53.6 \pm 1.2	0.78 \pm 0.13	1.6 \pm 0.3	17.1 \pm 1.4
5HUBM	0.006	47.5 \pm 1.9	2.04 \pm 0.24	4.7 \pm 0.6	51.8 \pm 3.9

Experimental conditions were the same as those in Table 1 except that NBM controls in the CFUC assay formed 150 colonies per 10^4 nucleated cells

CFUC per colony almost 2 times greater (M.R., unpublished results.)

The hypothesis predicts that CFUS from foetal liver will have greater capacity for self-renewal than those from NBM and that stem cells, such as those in the blood whose capacity for self-renewal is less than that of NBM⁹, can be shown to be "older". Preliminary results confirm that this is so.

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Cellular and humoral immune responses in vaccination against dental caries in monkeys

PROTECTION against dental caries in the rhesus monkey by immunisation has now been characterised with respect to the development of *Streptococcus mutans*¹ and the corresponding antibodies²⁻⁴. One of the essential requirements for protection is the development of an optimum level of antibodies, at a critical time, before a large enough number of *Strept. mutans* has developed to initiate caries formation². Antibody synthesis, amplification mechanisms and protection depend on cellular interactions associated with T and B lymphocytes and macrophages⁵. The aims of this investigation were to study the part that cell-mediated immune responses might play in the protective mechanism against dental caries. The incidence of caries and the growth of *Strept. mutans* in rhesus monkeys was studied sequentially and compared with the development of serum antibodies, *in vitro* DNA synthesis of antigen stimulated lymphocytes, leukocyte migration inhibition (LMI) and *in vivo* skin delayed hypersensitivity (DH) to *Strept. mutans*.

Eight young rhesus monkeys were caged, examined and maintained on a human type of diet, containing 15% sucrose⁶. All animals had a fully erupted deciduous dentition and the permanent first molars were erupted in four monkeys; in the other four the permanent first molars erupted into a functional position within 30 weeks of the start of the experiment. As younger animals tend to develop more caries^{7,8}, the distribution of the monkeys was biased against the immunised group by including three of the younger monkeys without the permanent molars. A strain of

Strept. mutans (serotype c) was isolated from the dental plaque of a rhesus monkey which was used as a control in a previous experiment¹. The organism was grown in Todd-Hewitt broth, killed with 0.6% formalin, and a vaccine containing 2×10^9 *Strept. mutans* per ml was prepared². The three youngest monkeys were given subcutaneous injections of the vaccine mixed with an equal volume of Freund's incomplete adjuvant, 0.5 ml was injected into the right arm and 0.5 ml into the left leg at the start of the experiment. The same amount of *Strept. mutans* in saline was administered subcutaneously 30 weeks later. The sham-immunised group of three monkeys was given subcutaneous injections of 0.5 ml of saline instead of the vaccine. A further control group of two monkeys had no injections.

The animals were examined clinically and by means of X-rays for the presence of dental caries, they were weighed and blood was taken from the femoral vessels at 3-weekly intervals. At the same time, dental plaque was collected with a probe from the cervical and approximal surfaces of the upper left deciduous molars. The two control groups showed a very similar increase in caries and the number of colony-forming units (CFU) of *Strept. mutans*, so that they were combined for statistical analysis. Smooth surface (SS) caries was detected from week 18 (Fig. 1) and the mean rate of increase in caries in the immunised animals was significantly less than in the controls ($P < 0.05$). At week 54 a mean of $8.4 (\pm 1.2)$ cavities per animal was found in the controls compared with 2.7

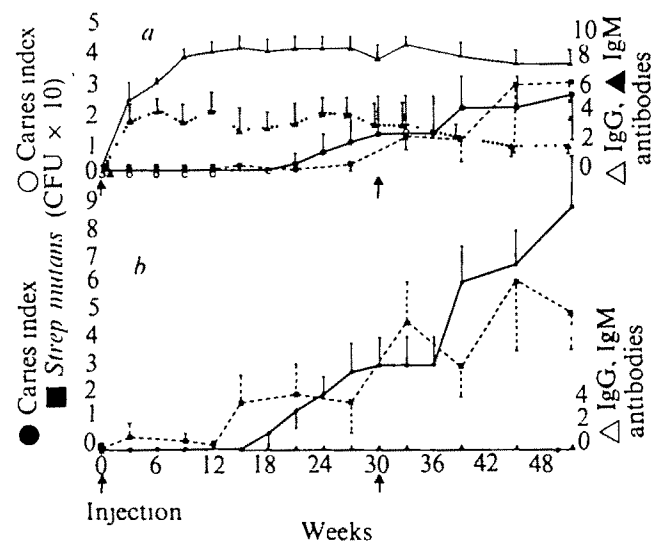


Fig. 1 Sequential analysis of caries, antibodies and *Strept. mutans* in monkeys immunised with *Strept. mutans*. For antibody titration¹⁸ doubling dilutions of sera were added to air-dried smears of cells of the immunising *Strept. mutans* for 30 min, washed three times with phosphate-buffered saline (PBS), and then overlaid with anti-human IgG (F.P. 3.1), or IgM (F.P. 4.1) fluorescein-isothiocyanate conjugates (Wellcome). The reagents were checked for class specificity and cross reactivity with monkey IgG and IgM and specificity of the test was established by complete absorption of the antibody titre with the homologous and little or no absorption with heterologous organisms. The titres were expressed as $\log_2(1=1/5)$. *Strept. mutans* CFU are expressed as the percentage of total anaerobic colonies on TYC agar¹⁹, when dental plaque from the upper left deciduous molars was cultured for 48 h. Caries index is the number of smooth surface carious lesions detectable clinically or by means of X-rays². a, Immunised, b, sham-immunised.

(± 1.8) SS cavities per animal in the immunised group. Only three fissure caries lesions appeared by 54 weeks in the deciduous teeth of two of the five controls and in none of the immunised monkeys.

The decrease in the incidence of dental caries is consistent with the results of previous immunisation studies¹, but there were two major differences in the experimental design. A formalin-killed *Strep. mutans* organism, isolated from a rhesus monkey, was used, unlike the heat-killed human strain of *Strep. mutans* in the previous studies. Furthermore, unlike the six immunising injections in the first study² and five injections in the second study¹, only two injections were used at an interval of 30 weeks. Indeed it is not certain that the boosting injection was necessary, as the IgG antibody titre at that stage had decreased by only one dilution step.

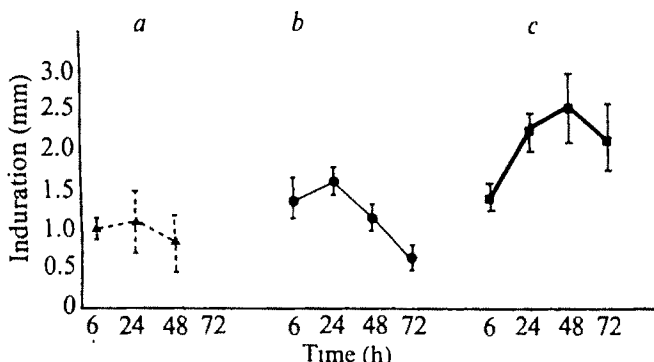


Fig. 2 Skin DH to *Strep. mutans*. A sample of 0.05 ml of Mickle disintegrated cells of *Strep. mutans* (10^9 cells per ml) or physiological saline was injected intradermally into the shaved abdominal wall of each animal. Skin induration was measured with calipers at the times stated and expressed as the mean specific increase in skin thickness (\pm s.e.m.) due to *Strep. mutans*, after deduction of the skin thickness of the site injected with saline. a, Pre-immunised; b, sham-immunised; c, immunised.

Cutaneous delayed hypersensitivity was measured 24 weeks after the second immunising injection (Fig. 2). The mean (\pm s.e.) of skin induration of the three serial skin tests in each group of animals is given in Fig. 2 only for the broken cells, as the whole cells and culture extract failed to induce significant skin DH reactions. The results show a slight antigenic toxicity, in that the pre-immunised monkeys showed an induration of $1.0 (\pm 0.1)$ mm at 6 h (Fig. 2). The induration did not increase at 24 h, however, and decreased at 48 h. The sham-immunised monkeys showed a slight increase in induration from $1.42 (\pm 0.25)$ mm at 6 h to $1.62 (\pm 0.16)$ mm at 24 h and then a decrease at 48 and 72 h. In the immunised monkeys there was a significant increase from $1.42 (\pm 0.17)$ at 6 h to $2.23 (\pm 0.23)$ at 24 h ($P < 0.02$), to $2.54 (\pm 0.45)$ at 48 h ($P < 0.05$) and a decrease to $2.2 (\pm 0.45)$ at 72 h; the latter just failed to reach the 5% level of significance compared with the response at 6 h. The results indicate a skin DH reaction which reaches maximal value at 24–48 h.

When the lymphoproliferative response of the monkeys was examined, lymphocytes from pre-immunised and sham-immunised monkeys failed to respond to any of the *Strep. mutans* preparations, so that these were combined into one control group (Table

Table 2 Lymphoproliferative responses of T and B lymphocytes

No	T and B		Lymphocytes		B	
	c.p.m.*	SI†	c.p.m.	SI	c.p.m.	SI
1	1,331	12.8	1,390	1.8	524	1.2
2	1,468	2.6	427	3.1	841	1.7
3	2,158	4.5	1,004	3.6	3,551	1.1
4	2,573	9.9	1,484	7.0	nd	
5	3,194	4.2	2,959	4.2	nd	
6	3,264	7.1	1,339	9.7	nd	
7	1,291	2.1	1,819	3.4	nd	
8	1,716	3.4	1,514	4.2	nd	
Mean	5.8		5.9			1.3
s.e.	1.34		1.2			0.2

Lymphocyte transformation was assessed as for Table 1. T lymphocytes were prepared by passing lymphocytes over nylon wool adherence columns¹⁶. B lymphocytes were obtained by depleting lymphocytes of cells rosetting with neuraminidase-treated ovine erythrocytes¹⁷, by Ficoll–Triosil gradient sedimentation. Cell purity was assessed by direct staining for surface immunoglobulin with fluorescein–isothiocyanate-labelled polyvalent anti-human Ig (Wellcome).

*No antigen

†*Strep. mutans* whole cell antigen

1). Significant differences between controls and immunised monkeys were found with the culture extract ($P < 0.001$), cell walls ($P < 0.01$) and whole cells ($P < 0.02$) of *Strep. mutans*, respectively, though only the whole cells yielded moderate stimulation index (SI). The cellular responses were limited to T lymphocytes, for 96–98% purified T cells yielded significant stimulation by antigens, whereas 94% enriched B lymphocytes failed to respond (Table 2).

Thus both the lymphoproliferative response and skin DH to the immunising antigens were found 24 weeks after the last immunisation, with a good correlation between the two tests for the experimental and control groups. In contrast, phytohaemagglutinin (PHA) did not show a difference between the immunised and control monkeys, but significantly increased SI to pokeweed mitogen (PWM) were found in the immunised monkeys ($P < 0.02$; Table 1). Although the differential response to the mitogens might have been due to the adjuvant, this is unlikely, as a comparison has been made between animals injected with saline and Freund's incomplete adjuvant (unpublished). There was no significant difference in the lymphoproliferative responses between the saline and adjuvant injected monkeys with PHA (5.7 ± 1.7 and 8.3 ± 3.4) and with PWM (10.5 ± 5.6 and 16.6 ± 8.8) respectively.

As the T- and B-cell mitogen (PWM), unlike the T-cell mitogen alone (PHA), showed a greater proliferative response in the immunised animals, this argues in favour of a heightened B-cell responsiveness in the immunised animals. This is consistent with the high antibody response maintained throughout the experimental period.

Serum IgG and IgM antibodies were assayed by the indirect fluorescent antibody (FA) test (Fig. 1). IgG antibodies increased within 3 weeks of immunisation to a mean \log_2 titre of $5.3 (\pm 1.2)$ which increased to an optimum titre of $\log_2 8.3 (\pm 0.35)$ by week 9 and this level was maintained throughout the experimental period. IgM antibodies were also increased but the titre was lower than that of IgG throughout the experimental period (Fig. 1). The titre

Table 1 Lymphocyte transformation induced by antigens and mitogens

Group	No	Mean c.p.m. (\pm s.e.) of unstimulated cultures	Mean (\pm s.e.) of SI to <i>Strep. mutans</i> antigens				Mitogens	
			Whole cells	Broken cells	Cell walls	Culture extract	PWM	PHA
Control	5	1,035 (± 357)	1.2 (± 0.2)	0.9 (± 0.2)	0.7 (± 0.1)	0.8 (± 0.2)	9.9 (± 3.9)	13.2 (± 5.6)
Immunised	3	863 (± 103)	5.9 (± 1.7)	2.2 (± 0.8)	2.0 (± 0.05)	2.7 (± 0.4)	33.8 (± 7.4)	12.2 (± 0.5)
t		-0.891	-3.623	1.941	8.785*	5.245	3.186	0.123
P		NS	<0.02	>0.05	<0.01	<0.001	<0.02	NS

1×10^6 Ficoll–Triosil separated lymphocytes, in 1.0 ml of 10% autologous serum in medium TC199 (Wellcome), were cultured for 120 h¹⁸. Antigens in previously determined optimal concentrations were added in 0.1-ml amounts. PHA (Wellcome) was used at $50 \mu\text{g}$ per culture and PWM (Gibco) at $10 \mu\text{g}$ per culture. Cells were labelled with ^3H -thymidine ($1 \mu\text{Ci}$ per 10^6 cells) for the last 18 h of culture and collected using an automatic sampling device. The c.p.m. of unstimulated cells are shown \pm s.e.m. and SI is the ratio between antigen-stimulated and control cultures. NS, not significant.

*Only five monkeys tested.

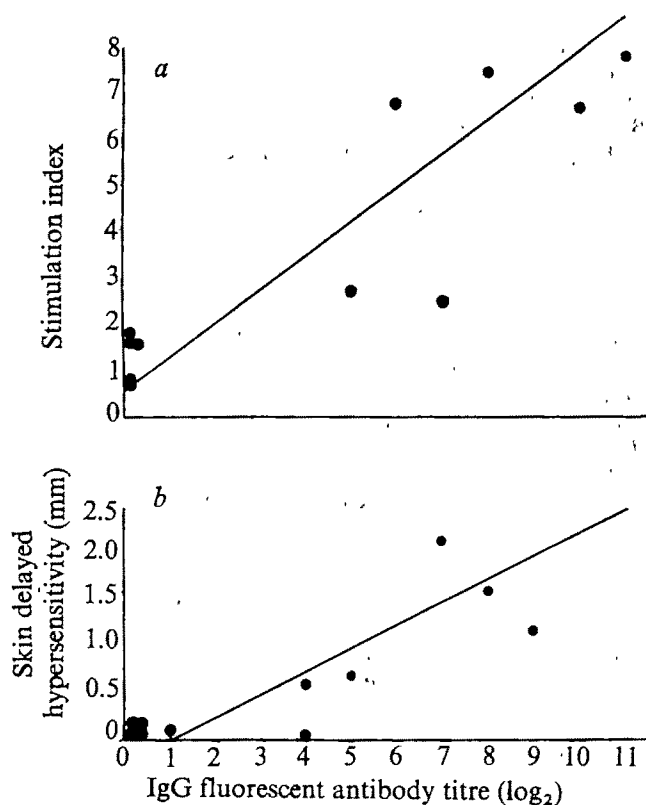


Fig. 3 Relationship between IgG antibodies to *Streptococcus mutans* and lymphocyte transformation (a) or skin DH (b). IgG fluorescent antibodies were assayed as described in Fig. 1. a, Lymphocyte transformation as in Table 1. SI is the ratio of ³H-thymidine uptake in *Streptococcus mutans* whole cell-stimulated and control cultures after 120 h of culture with 1×10^6 Ficoll-Triosil separated lymphocytes, in 10% autologous serum in medium TC199. b, Skin testing for DH as in Fig. 2. Results shown are mean specific increases in skin thickness 48 h after injection of *Streptococcus mutans* broken cells.

was maintained from weeks 3 to 30 at a level of about log₂ 3–4, but in spite of the second immunising injection at week 30, the titre fell to a level of less than log₂ 2 by week 45. None of the control sera showed IgG or IgM fluorescent antibodies.

The finding of significant skin DH as well as antibodies in immunised monkeys raised the question of whether the DH might be a measure of T-cell helper function⁹. To enable us to analyse linear regression with some confidence, three further monkeys previously immunised with *Streptococcus mutans* (group c) were included. A significant positive correlation ($r=0.857$, $P<0.001$) was found between the 48-h skin induration and the IgG fluorescent antibody titre (Fig. 3b) and this is consistent with a T-cell helper function in antibody formation. As with the skin DH reaction a significant positive correlation (Fig. 3a), was found between the proliferative response of lymphocytes and the IgG antibodies to *Streptococcus mutans* ($r=0.884$, $P<0.001$). IgM was neither correlated with the skin DH reaction ($r=0.0826$, $P>0.1$) nor with the lymphoproliferative response ($r=0.411$, $P>0.1$). The protective mechanism against dental caries may thus be dependent on the helper function of T lymphocytes in generating IgG antibodies, as has been found in other systems¹⁰.

As another measure of cellular responses, lymphokine production was assayed by the leukocyte migration inhibition test (LMIT)¹¹. The results failed to distinguish between the two groups, as both the immunised (three of three) and sham-immunised (two of three) groups yielded significant LMI tested with whole cells of *Streptococcus mutans* (Table 3). This finding raised the possibility that conversion of LMI in the control monkeys was induced by the overgrowth of *Streptococcus mutans* in the bacterial plaque, as two of three sham-immunised monkeys also gave a positive LMIT with broken cells of *Streptococcus mutans*. Indeed, the LMIT in preimmunised monkeys which had not been exposed to the human cariogenic diet and had little or no *Streptococcus mutans* was consistently negative, supporting the view that sensitisation to *Streptococcus mutans* may have been induced through the gingival crevicular epithelium or the gut, by the increased load of *Streptococcus mutans* in the bacterial plaque.

Sequential analysis of the CFU of *Streptococcus mutans*, revealed consistently larger numbers of *Streptococcus mutans* in the control than in the immunised animals (Fig. 1). The results suggested that *Streptococcus mutans* were quantitatively related to the number of carious lesions in the control as well as the immunised groups, and that both the caries index and the number of *Streptococcus mutans* were decreased by immunisation.

It is also likely that not only the *Streptococcus mutans* antigen but also the adjuvant was required, as proposed by the modified "two signal model" in which an antigen-induced mitotic stimulus will result in the formation of a clone of antibody-forming cells only in the presence of a second, adjuvant stimulus¹². Indeed, negligible antibody titres and no lymphoproliferative responses were found on immunisation with *Streptococcus mutans* cells, without an adjuvant (unpublished).

In view of the great technical difficulties in monkeys of thymectomy *in vivo* or cell depletion *in vitro*, these have not been carried out. It is therefore not possible to draw definite conclusions as to T- and B-cell cooperation, though the results are consistent with the hypothesis that protection against *Streptococcus mutans* in dental caries involves T- and B-cell cooperation^{13,14}. This is based on finding: (1) a significant positive correlation between IgG antibodies and the proliferative response of lymphocytes to *Streptococcus mutans*, the latter being a function of T lymphocytes, and (2) a significant positive correlation between IgG antibodies and the skin DH reaction which may be a measure of T-cell helper function⁹.

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Table 3 Leukocyte migration inhibition test

Group	No.	No. of positive*, and (mean \pm s.e.) of migration index to <i>Streptococcus mutans</i>		
		Whole cells	Broken cells	Culture extract
Sham-immunised	3	2 (81.3 \pm 12.3)	2 (64.3 \pm 19.9)	1 (79.3 \pm 11.9)
Immunised	3	3 (74 \pm 2.2)	0 (86.7 \pm 2)	1 (90 \pm 11.3)
Preimmunised	3	0 (105.7 \pm 7.5)	0 (131.3 \pm 2.3)	0 (140.7 \pm 27.2)

Leukocytes were separated from peripheral blood using 3% dextran, washed twice and adjusted to 1×10^7 per ml in 10% horse serum with medium TC199. After filling, the capillary tubes (20 μ l) were plugged and centrifuged at 250g for 5 min, cut at the cell-fluid interface and placed in 1.0 ml of antigen-containing medium for 18 h in plastic chambers. Migration areas were recorded and measured by compensating planimetry. Migration index is the ratio between the area of leukocyte migration with and without antigen.

*Migration index < 80.

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Ly phenotype of T-cells releasing T-cell replacing factor

THE Ly phenotype of T cells which cooperate with B cells in the production of antibody has been described as Ly 1⁺2⁻ (refs 1 and 2). Helper T cells are distinct from cytotoxic T cells (Ly 1⁻ Ly 2⁺)^{1,2} and suppressor T cells (Ly 1⁻ 2⁺)^{3,4}. In tissue culture, helper activity does not depend on the presence of intact T cells but can be mediated by cell-free supernatants from stimulated T cells. Stimulation with antigen induces the release of antigen-specific helper factors^{5,6} as well as factors which support antibody response against unrelated antigens⁷. Factors similar or identical to the latter are produced by splenic T cells in response to alloantigens⁸⁻¹⁰ and concanavalin A¹¹. It is tacitly assumed that the mechanism by which helper T cells collaborate with B cells in the production of antibody is to be attributed to the release of these mediators. We report here that with respect to their Ly phenotypes, T cells which release T-cell replacing factor (TRF)⁸ in response to alloantigen belong to the same subpopulation of T cells as helper cells.

In the experiments summarised in Table 1, TRF was generated in a one-way allogeneic reaction: spleen cells from C57BL/6 (B6) mice were cocultured with spleen cells from (B6×DBA/2)F₁ mice. The subpopulation of T cells responsible for TRF release was determined by cytolysis of

the B6 spleen cells with antisera directed against distinct T-cell alloantigens. Factor activity was assayed by its ability to restore antibody formation against sheep red blood cells (SRBCs) in cultures of T-cell depleted spleen cells from (B6×DBA/2)F₁ mice.

The production of TRF was completely abrogated after treatment with anti-Ly 1 serum or anti-Thy 1 serum and complement. Treatment by anti-Ly 2 serum and complement had no effect. Mixture of anti-Ly 2-treated cells with anti-Ly 1 or anti-Thy 1-treated populations, containing half as many cells of each kind compared with the unmixed populations, produced about 50% of the TRF activity achieved with untreated or anti-Ly 2-treated populations.

These data show that the presence of Ly 1⁺2⁻ T cells is sufficient for TRF production. Ly 1⁺2⁺ or Ly 1⁺2⁺ cells neither produce TRF nor contribute to the production of TRF.

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Table 1 Effect of antisera on TRF production

B6 strain	Cytolysis with antiserum	TRF production Ly phenotype of T cells tested for TRF production	Assay for TRF	
			BDF ₁ spleen cells	Anti-SRBC PFCs per culture (day 5)
B6 Ly 1.1 spleen	None	Ly 1 ⁺ 2 ⁻ ; Ly 1 ⁺ 2 ⁺ ; Ly 1 ⁻ 2 ⁺	T-cell depleted	550
(a)	Anti-Ly 1.1+C	Ly 1 ⁻ 2 ⁺		0
(b)	Anti-Ly 2.2+C	Ly 1 ⁺ 2 ⁻		670
(c)	Anti-Thy 1.2+C	None		0
Equal mixture of (a) and (b)		Ly 1 ⁺ 2 ⁻ ; Ly 1 ⁻ 2 ⁺		200
Equal mixture of (b) and (c)		Ly 1 ⁺ 2 ⁻		324
B6 Ly 2.1 spleen	None	Ly 1 ⁺ 2 ⁻ ; Ly 1 ⁺ 2 ⁺ ; Ly 1 ⁻ 2 ⁺		770
	Anti-Ly 1.1+C	"		700
	Anti-Ly 2.2+C	"		640
	Anti-Thy 1.2+C	None		0
No factors added			Untreated	5
				940

2 × 10⁷ spleen cells per ml were suspended in 1 ml of 1:6 diluted anti-Ly 1.1 ((BALB/c × B6)F₁ anti-B6/Ly 1.1 thymocytes, absorbed with B6 thymocytes) or anti-Ly 2.2 ((C3H × B6/Ly 2.1)F₁ anti-B6 leukaemia ERLD, absorbed with B6/Ly 2.1 thymocytes) or 1:10 diluted anti-Thy 1.2 serum ((A/Thy 1.1 × AKR/H-2b)F₁ anti-A strain leukaemia ASL 1) or left untreated. After incubation for 30 min on ice, cells were washed with medium RPMI 1640 supplemented with 1% foetal calf serum. They were then resuspended at 1.5 × 10⁷ cells per ml in 1:24 diluted selected rabbit complement and incubated for 40 min at 37 °C. Cells were washed twice in medium and resuspended in equal volumes of medium such that the density of the complement control was 1.0 × 10⁷ cells per ml. To ensure serological specificity of anti-Ly antisera, the relevant congenic strain combinations were chosen. In the experimental group, spleen cells from the mouse strain B6/Ly 1.1 were treated with anti-Ly 1.1 or anti-Ly 2.2 antisera and complement. In control groups spleen cells of mice of the congenic strain B6/Ly 2.1 were treated with the same antisera and complement. In further control experiments all T cells were eliminated with anti-Thy-1.2 serum and complement. For the production of TRF⁸, equal volumes of B6 congenic cells (treated or untreated) and (B6 × DBA/2) F₁ spleen cells were cultured in standard conditions¹¹. Supernatants were collected after 24 h, passed through 0.45-µm Millipore filters and stored at -70 °C. The helper effect was evaluated using T-cell depleted BDF₁ spleen cells sensitised with SRBC (basically following the Mishell-Dutton culture method¹¹). 0.2 ml of anti-Thy-1.2-treated BDF₁ spleen cells adjusted to a volume corresponding to 2 × 10⁷ cells per ml in the complement control were added to multiwell plates (Falcon No. 3008), and 0.1-ml samples of the allogeneic supernatants described above or culture medium were added 24 h later. Plaque forming cells (PFCs) were determined by the Jerne haemolytic plaque assay after culture for 5 d. The data given are from one representative experiment. Repeat experiments gave similar results.

Role of chloride transport in regulation of intracellular pH

It is well known that introducing acid into a cell causes a rapid fall in intracellular pH (pH_i) which is followed by a slower rise¹⁻⁵. Since this return of pH_i towards its original value could not be accounted for by the passive transmembrane movement of H^+ , OH^- , or HCO_3^- , it was ascribed to the active removal of acid from the cell^{1,2}. In the squid giant axon, this acid extrusion is reversibly blocked by cyanide and greatly enhanced by HCO_3^-/CO_2 (ref. 3). More recently, it has been found that acid extrusion in the snail neurone also is stimulated by HCO_3^-/CO_2 (ref. 4), and that, in addition, it is inhibited by 4-acetamido-4'-isothiocyano-stilbene-2,2'-disulphonic acid (SITS)⁵, a known inhibitor of anion fluxes in erythrocytes⁶. In this respect it is interesting to note that a component of Cl^- efflux in barnacle muscle also is stimulated by HCO_3^-/CO_2 and blocked by SITS⁷. We now report that acid extrusion in the squid axon requires internal Cl^- and ATP, that it is blocked by SITS, and that it is accompanied by the SITS-sensitive efflux of an equivalent amount of Cl^- . These observations suggest that acid extrusion actually involves the neutral exchange of external HCO_3^- for internal Cl^- .

Our experiments were carried out on giant axons of the squid *Loligo pealei*. To control the intracellular concentrations of low molecular weight solutes, we used the internal dialysis technique⁸: porous dialysis capillaries were introduced into cannulated axons, and flushed with solutions of known composition. In one series of experiments, pH_i was monitored by placing

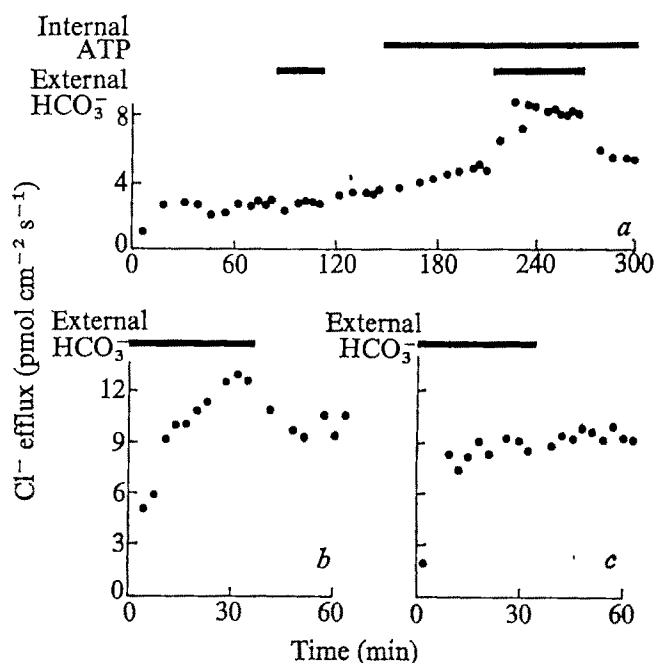
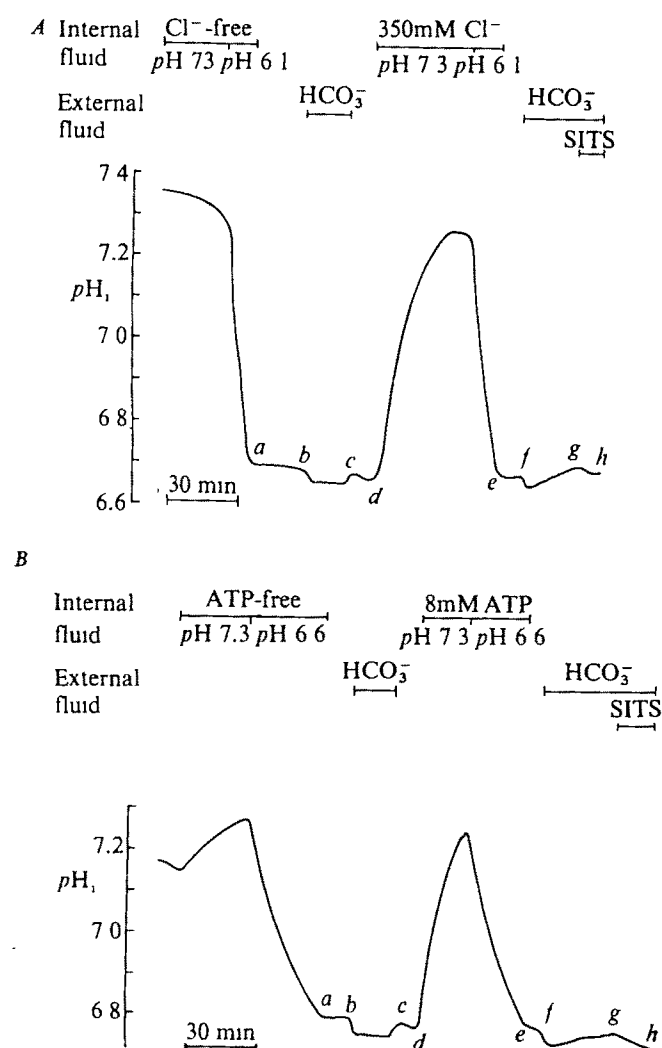


Fig. 2 $^{35}Cl^-$ efflux from internally dialysed squid axons. **a** ATP-dependence of HCO_3^- -stimulated Cl^- efflux. The axon was dialysed with a solution containing 150 mM Cl^- , and at zero time the flow of isotope-containing dialysis fluid was begun. The fluid superfusing the axon contained nominally 2 mM NaCN and was collected at 3- or 4-min intervals. An appropriate volume of a 2:1 toluene-Triton X-100 scintillation mixture was added¹⁰ and the samples counted long enough to achieve at least a 5% counting accuracy. There was no stimulation of $^{35}Cl^-$ efflux by 10 mM $HCO_3^-/0.4\% CO_2$ unless the dialysis fluid contained ATP. V_m was -60 mV at the beginning of this experiment, and -53 mV at the end. **b** and **c**, Effect of 0.5 mM SITS on HCO_3^-/CO_2 -stimulated Cl^- efflux. In the experiment represented in **b** $^{35}Cl^-$ efflux fell when the HCO_3^-/CO_2 was removed from the ASW ($V_m = -60$ mV). In **c**, however, where the axon had been pretreated for 50 min with 0.5 mM SITS, removal of the HCO_3^-/CO_2 had no effect ($V_m = -64$ mV). In four axons, 0.5 mM SITS caused the HCO_3^-/CO_2 -stimulated Cl^- efflux to decline from 10.1 ± 1.5 to 7.2 ± 1.0 pmol $cm^{-2} s^{-1}$. pH of dialysis solution 6.8; temperature 15 °C.

Fig. 1 **A**, Effect of $(Cl^-)_i$ on the acid extrusion. The axon was dialysed first with Cl^- -free (glutamate replacing Cl^-) and then with 350 mM Cl^- fluid, while pH_i was monitored with a glass electrode. In the absence of internal Cl^- , HCO_3^-/CO_2 (segment **bc**) did not stimulate acid extrusion ($n = 17$), whereas in the presence of 350 or 150 mM Cl^- (not shown), HCO_3^-/CO_2 (**fg**) did stimulate acid extrusion ($n = 14$). SITS 0.5 mM (**gh**) blocked the HCO_3^- -stimulated acid extrusion ($n = 7$). Membrane potential (V_m) was -57 mV at the outset of this experiment, and -47 mV at the end. The period of dialysis was always at least 40 min, a length of time sufficient to attain isotopic equilibration with $^{35}Cl^-$ (see Fig. 2). In the low Cl^- ASW, NaCl and KCl were replaced by sodium isethionate and $MgCl_2$ by $MgSO_4$. **B**, ATP dependence of acid extrusion. The axon, pretreated for 1 h in nominally 2 mM NaCN ASW, was dialysed first with an ATP-free and then with an 8 mM ATP solution (150 mM Cl^-). Although HCO_3^-/CO_2 failed to stimulate acid extrusion in the absence of internal ATP (**bc**), HCO_3^-/CO_2 did stimulate this extrusion when ATP (**fg**) was present ($n = 3$). The acidification during **ef**, which probably reflects breakdown of the exogenous ATP, was minimised by pretreating the axon with 10^{-6} M ouabain and by including oligomycin ($12 \mu g ml^{-1}$) in the dialysis fluid. In this experiment, the initial and final V_m was -61 mV. Temperature of both experiments 22 °C. The pH of the 10 mM $HCO_3^-/0.4\% CO_2$ ASW was 8.0; all other ASWs in this study had a pH of 7.7. Unless otherwise noted, the composition of the dialysis fluid was (mM): 150 KCl, 200 K glutamate, 50 Na glutamate, 4 $MgSO_4$, 190 taurine, 0.5 EGTA, 10 HEPES and 0.5 phenol red.

pH-sensitive and reference microelectrodes into the axon² alongside the dialysis capillary. In the other series, Cl⁻ efflux was measured in axons dialysed with solutions containing ³⁶Cl⁻.

The dependence of acid extrusion on internal Cl⁻ was investigated by measuring this extrusion, both in the presence and absence of internal Cl⁻. The axon of Fig 1A was superfused with low (20 mM) Cl⁻ seawater, and was first dialysed with a Cl⁻-free solution (pH 7.3). Acid was then introduced into the axon by lowering the pH of the dialysis fluid to 6.1. When the pH_i had fallen to 6.7 (point *a*), the flow of dialysis fluid was halted so that the subsequent course of pH_i was determined by cellular processes. In a normal axon, this fall in pH_i (acid challenge) would be followed by a rise, the rate of this alkalisation being an index of acid extrusion. In this axon depleted of internal Cl⁻, however, pH_i continued to fall (segment *ab*). Moreover, HCO₃⁻/CO₂ failed to reverse this decline (*bc*). In contrast, when Cl⁻ was reintroduced into the axon by dialysing with a 350 mM Cl⁻ solution, and the pH_i was once more reduced to 6.7 (*e*), HCO₃⁻-stimulated acid extrusion was observed (*ef*). Dialysis with 150 mM Cl⁻ gave identical results (not shown). Treating the axon with 0.5 mM SITS (*gh*) blocked the HCO₃⁻-stimulated acid extrusion.

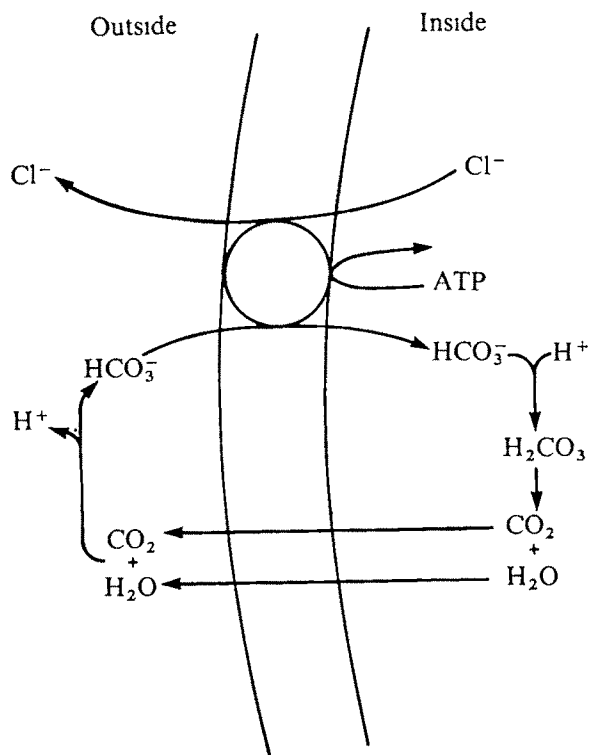


Fig. 3 Proposed scheme for acid extrusion. An ATP-dependent mechanism would inject external HCO₃⁻ in exchange for internal Cl⁻. Once inside, the HCO₃⁻ would combine with H⁺ to produce CO₂ + H₂O, which would then leave the cell and yield H⁺ + HCO₃⁻ once again. The net effect is to eject HCl from the cell.

The previously described inhibition of acid extrusion by cyanide³ suggested that acid extrusion might require ATP. One can reduce internal ATP to very low levels by dialysing with an ATP-free solution in the presence of cyanide⁹. In this manner, we lowered (ATP), in the axon of Fig 1B, and then challenged the axon with acid. No alkalisation (that is, no acid extrusion) was observed when dialysis was halted (*a*) even in the presence of HCO₃⁻/CO₂ (*bc*). When the axon was dialysed with an ATP-containing solution (*d*) in the continued presence of cyanide and then challenged with acid, the pH_i continued to fall (*ef*) after cessation of dialysis (possibly due to breakdown of exogenous ATP). But this decline was reversed when HCO₃⁻/CO₂ was added (*fg*). SITS again blocked this acid extrusion (*gh*), causing pH_i to fall at about the same rate as in segment *ef*.

The preceding experiments show that internal Cl⁻ and ATP are required for HCO₃⁻-stimulated, SITS-sensitive acid extrusion. The evidence that externally applied HCO₃⁻/CO₂ enhances Cl⁻ efflux from barnacle muscle, and that this HCO₃⁻-stimulated efflux is similarly SITS sensitive⁷ prompted us to consider the possibility that Cl⁻ efflux is linked to acid extrusion. We found that at relatively high pH_i, adding 10 mM HCO₃⁻/0.4% CO₂ had very little effect on acid extrusion. Similarly, we found that in these conditions (pH_i 7.3), 10 mM HCO₃⁻/0.4% CO₂ does not significantly increase Cl⁻ efflux. When the pH_i was lowered to about 6.7 by dialysis, however, both acid extrusion (Fig. 1) and Cl⁻ efflux were found to be enhanced by HCO₃⁻/CO₂: in 12 experiments 10 mM HCO₃⁻/0.4% CO₂ increased Cl⁻ efflux from 7.7 ± 0.6 (s.e.m.) to 11.6 ± 1.0 pmol cm⁻² s⁻¹ (15 °C). The response of Cl⁻ efflux and acid extrusion to removal of ATP provides another point of comparison between the two processes. As was the case with acid extrusion (Fig. 1B), Cl⁻ efflux did not respond to HCO₃⁻/CO₂ when the axon was deprived of ATP (Fig. 2a); responsiveness was restored when ATP was added to the dialysis fluid. In addition, just as with acid extrusion, HCO₃⁻/CO₂ stimulation of Cl⁻ efflux was inhibited by SITS, as can be seen by comparing Fig. 2b and c.

Thus, both HCO₃⁻-stimulated acid extrusion and Cl⁻ efflux require ATP and are blocked by SITS. In three acid extrusion experiments in which the temperature was the same as in the Cl⁻ studies (15 °C), HCO₃⁻-stimulated acid extrusion was estimated to be 4.8 ± 0.9 pmol cm⁻² s⁻¹, rather close to the HCO₃⁻-stimulated Cl⁻ efflux (3.9 pmol cm⁻² s⁻¹). It is attractive to hypothesise (Fig. 3) that the squid axon possesses an ATP-dependent pump which responds to acid challenges by taking up HCO₃⁻ from the external solution and extruding an equivalent amount of Cl⁻. Since the entering HCO₃⁻ is converted to CO₂ and H₂O, both of which subsequently leave the axon, the net effect of this transport mechanism is to extrude HCl.

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Acetylcholine receptor degradation measured by pulse chase labelling

THERE is increasing evidence that the turnover of the cholinergic receptor protein in developing or adult muscle fibres varies in the course of synaptogenesis or as a consequence of denervation^{1,2}. Muscle activity is involved in this regulation³, but the mechanisms of the control processes and the chemical signals which mediate these effects remain largely unknown.

To investigate this question several methods have been developed for the measurement of acetylcholine-receptor (AChR) turnover in embryonic muscle in tissue culture⁴ and denervated or neonatal rat diaphragm⁵. We present

here quantitative methods for the direct measurement of AChR turnover which depend on the incorporation of the radioactive precursor ^{35}S -L-methionine into newly synthesised receptor polypeptides and subsequently purification of radioactive receptor by affinity chromatography (ref. 4 and manuscript in preparation). These techniques have been applied to the determination of receptor half life in primary tissue cultures of foetal calf skeletal muscle. Results reported here indicate that AChR polypeptides with toxin binding activity have a half life of about 17 h. This value is identical to that obtained indirectly by measurement of the degradation of ^{125}I - α -bungarotoxin (^{125}I -BuTx) bound to receptor sites.

To measure directly the degradation of AChR, cultures of dissociated foetal calf skeletal muscle cells were pulse labelled with ^{35}S -L-methionine (10 Ci mmol^{-1} , CEA, France) for 7 h, 90 h after establishment of the cultures^{4,5}. After washing, the cells were chased with myoblast conditioned medium supplemented with $5 \times 10^{-5}\text{ M}$ L-methionine. At different times after the chase, 10 culture dishes were washed with warmed phosphate buffer saline (PBS) and stored at -80°C . AChR was purified from labelled cells by techniques described previously⁴ with the modifications indicated in the legend to Table 1.

Table 1 is a summary of AChR purifications from two pulse chase experiments. In every case, receptor activity (as determined by ^{125}I -BuTx binding assay⁶) from the labelled extract was quantitatively adsorbed to the affinity support of Erabutoxin-b coupled to Sepharose 4B (data not shown). The yield of each purification was therefore dependent on the efficiency of specific elution by the cholinergic agonist decamethonium. As can be seen in column C, Table 1, the yield or % recovery of toxin binding sites from different purifications varied from 11 to 29%. The observed variability is attributed to variation in specific elution from the affinity support. Recent experiments have shown that the receptor not recovered by decamethonium elution remains bound to the affinity support, suggesting it is not recovered in an inactive form.

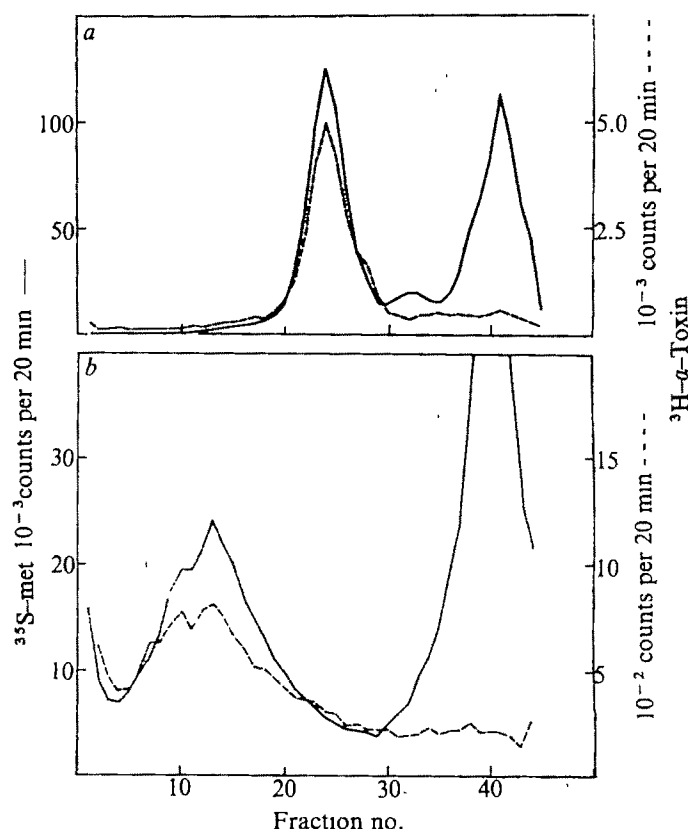


Fig. 1. Column purified receptor activity, after removal of decamethonium on DE-52 cellulose, was incubated at 4°C with ^3H - α -toxin from *N. nigricolis*, and normal or anti-*E. electricus* acetylcholine receptor rabbit serum. These incubations were centrifuged in isokinetic 10–40% sucrose gradients for 24 h at 40,000 r.p.m. in Beckman SW 41 rotor^{4,12}. Fractions were collected from the bottom of the tube and counted in Triton containing scintillation cocktail¹³ for ^3H (solid line) and ^{35}S (dashed line).

Table 1 AChR purification from pulse chase labelled cells.

Hours after chase	A pmol ^{125}I -BuTx sites adsorbed	B pmol ^{125}I -BuTx sites recovered	C ^{125}I -BuTx sites % recovery	D Total ^{35}S -met c.p.m.	E AChR ^{35}S -met c.p.m.	F Corrected AChR ^{35}S -met c.p.m.
Experiment 1						
0	30	3.5	11.4	5.48×10^4	4.07×10^4	3.58×10^4
14	34	9.9	29.1	4.25	5.40	1.85
23	34	8.9	26.1	3.80	2.99	1.15
Experiment 2						
0	30	5.9	19.3	4.45	1.37	7.1×10^4
16.5	39	5.9	15.0	3.54	0.63	4.2
28	26	5.9	22.7	3.10	0.61	2.7
40	24	5.9	25.0	2.42	0.30	1.2

Cells were labelled as described in the text. Frozen cells were scraped from the plates in distilled water. The water extracts were centrifuged at $100,000g$ for 30 min. The membrane pellet was suspended in 1% Triton X-100 in 0.18 M NaCl, 0.025 M, NaH_2PO_4 , pH 7.6 containing 10^{-4} M phenylmethylsulphonyl fluoride, 10^{-4} M benzethonium chloride and 10^{-3} M EDTA to inhibit protease activity¹⁴. After extraction for 2 h at 4°C , the extract was centrifuged at $100,000g$ for 30 min. The supernatant was removed and filtered through a plug of glass wool. Triton extracts were adsorbed to an excess of affinity support, consisting of Erabutoxin b, coupled to cyanogen bromide, activated Sepharose 4B ($100\text{ }\mu\text{g ml}^{-1}$)¹⁴. Adsorption was complete ($>80\%$) in 3–5 h. After the complete adsorption of receptor activity from the ^{35}S -labelled extracts, the affinity columns were incubated with an excess of cold crude extract containing receptor. Receptor adsorption was monitored by the ^{125}I -BuTx binding assay. Column A is the amount of total toxin-binding activity. (^{35}S labelled plus cold) bound to the column. The columns were washed with 0.36 M NaCl, 0.05 M NaH_2PO_4 , pH 7.6, 1% Triton, until the level of ^{35}S radioactivity in the wash was reduced to background. Toxin binding activity was released from the column by incubation with 0.1 M decamethonium at 4°C for 40 h. After dilution, the toxin column eluates were adsorbed to and eluted from DE-52 cellulose columns to remove decamethonium. The toxin-binding activity was then assayed by ^{125}I -BuTx binding. The yield of each purification was calculated from the toxin-binding activity recovered relative to that initially bound, column C. Total ^{35}S -methionine c.p.m., column D, was determined by hot TCA precipitation of an aliquot of the crude water extract. The amount of AChR ^{35}S -methionine c.p.m. was determined by analysis of the toxin column purified material (after DE-52 chromatography) on sucrose gradients. Complete recovery of input ^{35}S c.p.m. was obtained. Only the 9S toxin binding peak was summed and recorded in column E. Finally, these values were corrected to 100% recovery, column F.

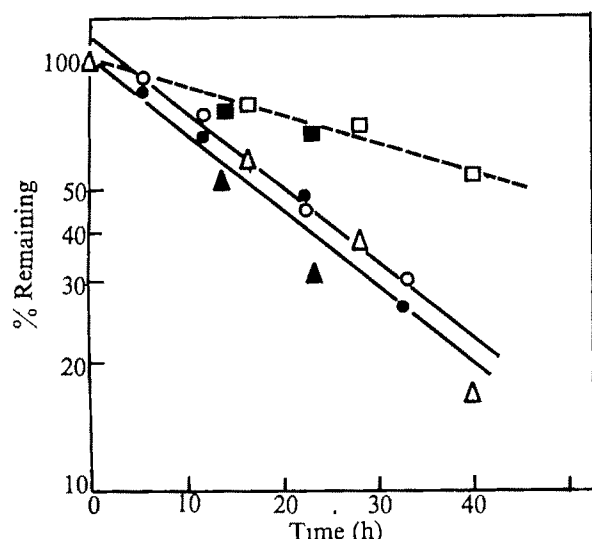


Fig. 2 AChR degradation. The corrected values for AChR 35 S-c.p.m. remaining after the chase from Table 1 were normalised to a zero time 100% figure and plotted here as a first-order decay as open and filled triangles for the two separate experiments. The total protein 35 S-c.p.m. was similarly normalised and plotted as open and closed squares. The loss of bound 125 I-BuTx was studied as described by Devreotes and Fambrough⁴. Cells were incubated with 125 I-BuTx (prepared as described by Vogel *et al.*, 1972 (ref. 13) overnight at a concentration of 5×10^{-9} M. Unbound toxin was washed free by six successive washes with warmed complete culture medium. Finally, cells were returned to the culture with complete myoblast conditioned medium. At different times, four labelled plates were washed several times with PBS and scraped in 1% Triton X-100, 0.18 M NaCl, 0.025 M NaH_2PO_4 (pH 7.6). These extracts were counted directly in a gamma counter. All values from two independent experiments were normalised to a zero time of 100% and were plotted as open and closed circles.

At each step of the purification, the 35 S-methionine content of the sample was determined by scintillation counting. Column D, Table 1, lists the total hot trichloroacetic acid precipitable material. In column E is recorded the summed 35 S-methionine radioactivity of the affinity purified receptor which was observed to cosediment with the 9S peak of toxin-binding activity in a 10–40% isokinetic sucrose gradient^{1,12}. The fraction of 35 S-c.p.m. thus sedimenting represented 63–83% of the total in the gradient and was quantitatively shifted to a more rapidly sedimenting form by preincubation with rabbit antiserum prepared against purified AChR from *Electrophorus electricus*. One example of such an analysis is shown in Fig. 1 (ref. 4). Furthermore, the affinity purified material was found to be of high purity by two-dimensional high resolution gel electrophoresis (ref. 7 and J.P.M., manuscript in preparation).

The values obtained by sucrose gradient analysis were finally corrected for variation in the yield and are recorded in column F. It can be calculated that the synthesis of ACh receptor makes up only a very small fraction of global protein synthesis, about 0.065% in experiment 1 and 0.016% in experiment 2.

To calculate a receptor half life from the experimental data in Table 1, the values in column F were normalised to a zero time figure of 100%. In a similar fashion, a mean half time was calculated for total protein values in column D. The respective half lives were 17 h for the AChR and 48 h for total protein.

The value of 17 h for ACh-receptor half life by direct pulse chase experiments is in good agreement with values previously published for AChR from chick skeletal myotubes in cultures^{1,8} and extra synaptic receptor of denervated rat diaphragm^{2,9}. All of these previous measurements have been made by the indirect method in which the

degradation of bound 125 I-BuTx is observed. We have confirmed the half-life measurements by the indirect method in our tissue culture system and have found a value of 17 h, identical to that determined by the pulse chase experiments. This agreement lends support to the argument that the toxin-binding method is a valid probe of receptor metabolism^{2,9}.

From studies of loss of bound toxin to denervated or embryonic rat diaphragm, two rates of receptor degradation have been suggested^{9,10}. One, extrasynaptic, with a relatively high rate of turnover having a T_1 of 8–22 h, and a second more stable, synaptic, species with $T_1 > 100$ h. The reason for this considerable difference in degradation rate is not known. Furthermore, there is indirect evidence that the activity dependent alteration of extrasynaptic receptor levels is regulated at the level of polypeptide synthesis^{11,12}. Quantitative studies of receptor synthesis making use of the technique applied here to receptor degradation should permit the elucidation of the control mechanisms involved in the steady-state level of ACh receptor. It will be possible to determine how nerve-induced muscle activity functions to signal alterations in macromolecular synthesis, at what level (transcriptional or translational) is receptor synthesis controlled and what are the precursor-product relationships between synaptic and extrasynaptic receptor.

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Anticholinergic and membrane activities of amantadine in neuromuscular transmission

AMANTADINE hydrochloride (1-adamantanamine hydrochloride, Symmetrel) is an antiviral agent which prevents certain viruses from penetrating cells^{1–3}. In addition, this drug is also effective in relieving clinical symptoms shown in parkinsonism^{4–8}; this action of amantadine may depend on its capacity to increase the synthesis and release of dopamine from dopaminergic cells of the basal ganglia^{9,10}. To obtain additional evidence which might aid in explaining

these remarkably diverse effects of amantadine, we used single-cell electrophysiological techniques to study its action on junctional and extrajunctional membranes. Our results show that amantadine, in clinically effective concentrations, rapidly inhibits neuromuscular transmission, and when applied over a longer period of time the drug also exerts a substantial effect on the conductile membranes of muscle fibres.

In the first series of experiments we studied the action of amantadine on the indirectly-stimulated nerve-muscle preparation. Frog (*Rana pipiens*) sartorius muscle-sciatic nerve preparations were bathed in HEPES-buffered Ringer solution (112.4 mM Na⁺, 2.5 mM K⁺, 1.8 mM Ca²⁺, 117.1 mM Cl⁻, 3.0 mM HEPES buffer, pH 7.4) bubbled with 100% oxygen and containing dextrose (1 g l⁻¹). The sciatic nerve was stimulated electrically (0.1 ms min⁻¹) at a level 2.5 times that required for initiating the maximal isometric tension output. When the tension output had become constant, the preparation was curarised in two stages to produce a stable partial neuromuscular block¹¹. The nerve and muscle were first soaked for 10 min in HEPES-buffered Ringer solution containing 14 μ M *d*-tubocurarine (*d*TC) after which the *d*TC concentration was reduced to 0.7 μ M for the remainder of the experiment. Amantadine was added to the bath only after muscle tension output in the *d*TC-treated preparation had been stable for at least 60 min. For experiments where resting potentials (r.p.s), action potentials (a.p.s), endplate potentials (e.p.p.s) and miniature endplate potentials (m.e.p.p.s) were recorded intracellularly, the cutaneous pectoris nerve-muscle preparation was used *in vitro*. The preparation was bathed in HEPES-buffer Ringer solution, and where e.p.p.s were recorded, the two-stage curarisation technique described above was used. All experiments were done at room temperature (19–25 °C).

The results of isometric tension output measurements in partially curarised muscle are shown in Fig. 1. It can be seen that application of amantadine produced a simple reduction tension output which was reversed when this drug was removed. In three additional experiments, amantadine was applied to non-curarised nerve-muscle preparations. At 800 μ M amantadine, the tension output was reduced to 35% of control after 40 min exposure to the drug. At 500 μ M amantadine, the tension output fell to 60% of control after 55 min of drug application. Amantadine at 150 μ M did not produce a reduction in muscle tension output.

In the next series of experiments, e.p.p.s were recorded intracellularly from single muscle fibres of the curarised cutaneous pectoris muscle while nerve stimuli were applied once every 10 s. After a 30-s control period, for which three e.p.p.s were recorded and averaged, the junctional region was microperfused with bathing solution containing either 50 or 150 μ M amantadine. As shown in Fig. 2, the e.p.p.s were immediately and progressively reduced in

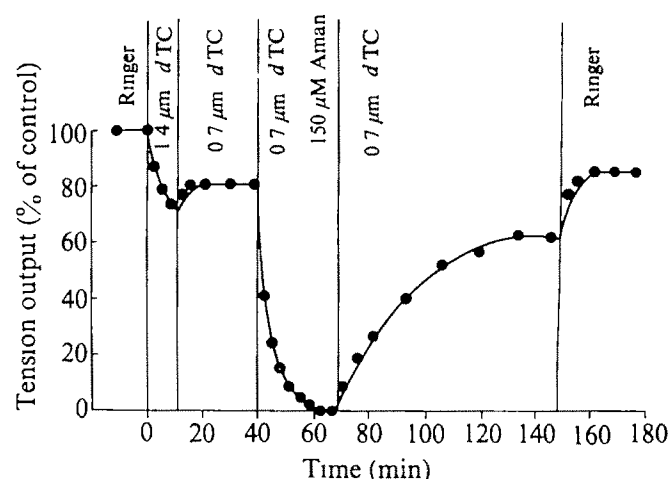


Fig. 1 Effect of amantadine on the isometric tension output of partially curarised, indirectly-stimulated sartorius muscle-sciatic nerve preparation of frog. Average of three muscles. Aman, amantadine.

amplitude following application of amantadine. During the 8-min period following cessation of amantadine application, the e.p.p. amplitude rose but did not reach the control value. The incomplete recovery is partially the result of a 10–12-mV fall in r.p. which occurred during the recording because the muscle twitch was not completely blocked in these experiments. More complete recovery would probably have occurred if additional time had been allowed, and if the muscle had been washed with amantadine-free bathing solution.

In the next group of experiments we showed that the slow reduction of resting potential observed in the previous experiments is not produced as a result of a postjunctional depolarising action of amantadine. The nerve-muscle preparation was treated as before with HEPES-Ringer solution plus *d*TC (two stages) but in this case only one e.p.p. was recorded from each individual muscle fibre of the sample group. Thereafter the muscle was bathed in HEPES-Ringer solution containing *d*TC plus amantadine (50 or 150 μ M) and a single e.p.p. was recorded from an additional group of individual fibres over a period of the next 30 min. The results of such experiments are summarised in Table 1. They show that there is a rapid reduction in the amplitude of the e.p.p. to 40% of control during the first 5 min of exposure to 50 μ M amantadine, and a reduction to 10% of control follows 5 min of exposure to 150 μ M amantadine. It can be seen that amantadine progressively depresses the amplitude of the e.p.p. during the entire 30-min period of its application, and it is also evident that amantadine does not depolarise the postjunctional membrane under these conditions.

Table 1 Effect of amantadine on resting potential and endplate potentials of partially curarised frog muscle fibres

No. of muscles	Amantadine (μ M)	Duration of soaking (min)	r.p. (mV)	n	e.p.p. (mV)	n
3	Control	—	91.8 ± 0.37*	49	11.9 ± 0.52*	49
	50 μ M	1–5	90.0 ± 0.67	14	4.7 ± 0.74	14
	50 μ M	5–10	90.5 ± 1.04	9	2.8 ± 0.74	9
	50 μ M	10–20	92.0 ± 1.36	8	1.7 ± 0.60	8
	50 μ M	20–30	91.3 ± 1.37	13	1.2 ± 0.20	13
2	Control	—	91.5 ± 0.44	28	10.2 ± 0.82	28
	150 μ M	1–5	93.0 ± 1.06	9	0.95 ± 0.26	9
	150 μ M	5–10	92.2 ± 0.67	10	0.66 ± 0.19	10
	150 μ M	10–20	92.0 ± 0.49	13	0.70 ± 0.17	13
	150 μ M	20–30	94.0 ± 0.77	8	0.38 ± 0.14	8

* Standard error of mean.
n, Number of fibres.

The local perfusion technique was also employed to observe the effects of amantadine on the amplitude and frequency of m.e.p.p.s. Microperfusion of two endplates with Ringer solution containing 300 μ M amantadine reduced the m.e.p.p. amplitude to zero in 12–48 s and the amplitude recovered to 70–85% of control 7 min after amantadine application had ceased. With 150 μ M amantadine, the m.e.p.p. amplitude in three endplates fell to 40–55% of control in 1 min and recovery was 65–90% complete in 5–7 min. At 50 μ M amantadine the m.e.p.p. amplitude in two endplates was reduced to 50% in 2 min and recovered to 70–80% in 4–7 min. The time course for depression and recovery of m.e.p.p. amplitude observed in these experiments was similar to that shown by e.p.p.s in the previous experiments. Amantadine at 50 and 150 μ M seemed to reduce the m.e.p.p. frequency but this limited observation needs verification, partly because amantadine reduces m.e.p.p. amplitude and thus the frequency count may be inaccurate because some low amplitude m.e.p.p.s may have gone undetected in the baseline noise of our recordings.

We have so far provided evidence that amantadine at 50 and 150 μ M has significant blocking action at the frog neuromuscular junction since it causes reduction in the tension output of the indirectly stimulated, partially curarised nerve-muscle preparation, and it also substantially reduces the amplitudes of e.p.p.s and m.e.p.p.s. The reduction in the amplitudes of m.e.p.p.s and e.p.p.s may be interpreted on the basis that amantadine reduces the sensitivity of the postjunctional membrane to acetylcholine and the following experiments were carried out to test this supposition. Local perfusion of nine junctions (three muscles) with 100 μ M amantadine reduced the m.e.p.p. amplitude from a control value of 0.35 mV to 0.11 mV in ~ 25 s. The m.e.p.p. frequency (min^{-1}) fell from 37 (control) to 15. In seven of these perfused junctions which were tested for response to nerve stimulation, five showed block of neuromuscular transmission (average e.p.p. amplitude 18 mV). During iontophoretic application of 5-ms pulses of carbamylcholine to the postjunctional membrane, microperfusion with 100 μ M amantadine reduced the carb depolarisations from 2.1 mV (control) to 0.3 mV in ~ 70 s (nine fibres). Thus the reduction in postjunctional sensitivity produced by amantadine is sufficient to account for its rapid action in reducing the amplitude of m.e.p.p.s and e.p.p.s and hence in causing a block in neuromuscular transmission.

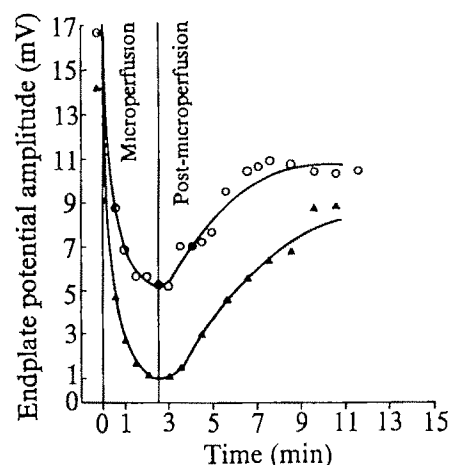


Fig. 2 Effect of amantadine on the amplitudes of endplate potentials recorded from partially curarised muscle fibres. ○, average of five endplates, 50 μ M amantadine; ▲, average of six endplates, 150 μ M amantadine.

In the final series of experiments, we investigated the direct action of amantadine on the muscle fibre conductile membrane. No change in r.p. or a.p. was produced during the first 30 min of application of 150 μ M amantadine. After 1 h of exposure to 150 μ M amantadine, however, depolarisation of the muscle fibres was evident and thereafter the depolarisation increased steadily with time. From Table 2, it may be seen that the average reduction of the resting potential was 8 mV between the first and second hour, and 18 mV during the second to third hour of exposure to 150 μ M amantadine. In addition, the amplitude, rate of rise and rate of fall of the a.p. were significantly reduced by application of 150 μ M amantadine for these time periods.

The results of our study indicate that amantadine rapidly inhibits neuromuscular transmission by reducing the response of the muscle postjunctional membrane to acetylcholine. When applied for relatively prolonged periods, it affects the resting and action potentials of extrajunctional excitable membranes. If amantadine produces similar changes in the resting and action potentials of the pre-synaptic neuronal membrane, one might predict that this drug would reduce the quantal output per nerve impulse. Unfortunately, it is not feasible to test this speculation by using the usual experimental methods because amantadine strongly inhibits postjunctional cholinergic receptors.

Table 2 Effect of 150 μ M amantadine on junctional and extrajunctional resting potentials and action potentials of frog muscle fibres

Conditions	n	r.p. (mV)	a.p. (mV)	m.r.r. (V s^{-1})	m.r.f. (V s^{-1})	Duration† (ms)	e.p.p.r.r. (V s^{-1})
Extrajunctional							
Ringer	12	91.7 \pm 0.41*	126.0 \pm 0.94*	505.0 \pm 12.40*	134.1 \pm 2.28*	0.80 \pm 0.008*	—
Amantadine	11	83.2 \pm 0.81	117.6 \pm 1.09	419.0 \pm 15.08	80.9 \pm 2.84	1.05 \pm 0.01	—
1st–2nd hour							
Amantadine	11	73.3 \pm 1.71	102.0 \pm 3.85	306.0 \pm 35.78	71.8 \pm 4.22	1.00 \pm 0.02	—
2nd–3rd hour							
Return to Ringer	12	87.0 \pm 0.85	123.0 \pm 1.29	465.4 \pm 15.51	117.5 \pm 2.78	0.88 \pm 0.01	—
15–45 min							
Junctional							
Ringer	6	89.3 \pm 1.42	119.0 \pm 2.03	648.3 \pm 31.56	81.6 \pm 5.42	0.80 \pm 0.04	191.6 \pm 15.79
Amantadine	12	79.6 \pm 2.08	110.8 \pm 3.19	530.0 \pm 37.63	54.5 \pm 5.54	1.10 \pm 0.01	65.3 \pm 8.66
1st–3rd hour							

*, Standard error of mean.

r.p., Resting potential.

n, Number of records.

†, Duration of a.p., measured at 0 mV line.

m.r.r., Maximal rate of rise of a.p.

a.p., Action potential.

m.r.f., Maximal rate of fall of a.p.

e.p.p.r.r., Maximal rate of rise of endplate potential.

We suggest that the beneficial effect of amantadine in parkinsonism may depend to some extent on the capacity of this drug to inhibit cholinergic transmission in certain neuronal circuits involved in the central control of muscular movement. The demonstrated action of amantadine on extrajunctional membranes may provide a route whereby the mode of action of this drug in preventing penetration of cells by viruses may ultimately be understood.

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Evidence for a prejunctional role of cyclic nucleotides in neuromuscular transmission

THERE is evidence for¹⁻³ and against^{4,5} the involvement of cyclic nucleotides in the release of neurotransmitters. We have investigated this question using a method more suited to the detection of prejunctional drug actions than those used previously. Instead of trying to infer the action of nucleotides on nerve endings by recording endplate potentials or muscle contractions *in vitro*, we recorded from single motor axons of cat soleus nerves *in vivo*. We found that dibutyl (db) cyclic AMP initiated activity in unstimulated motor axons and produced stimulus-bound AMP, db cyclic GMP, sodium butyrate and 5' AMP had no repetitive activity (SBR) in stimulated axons, whereas cyclic repeat. NaF and theophylline also initiated activity in unstimulated axons and produced SBR in stimulated axons. Pretreatment with the theophylline potentiated the effects of db cyclic AMP or NaF. The results suggest that cyclic AMP is involved in the excitation-secretion sequence of mammalian motor nerve endings.

The preparations have been described before.⁶ Cats were anaesthetised with α -chloralose (70 mg kg⁻¹), the popliteal fossa was dissected and all branches of the tibial nerve except that to the soleus muscle were cut. Similarly, all branches of the popliteal artery except the posterior tibial were occluded. Both heads of the gastrocnemius muscle were removed and the tendon of the soleus muscle was attached to a strain gauge. Dorsal laminectomy (L4-S1) exposed the ventral root of spinal nerve L7, which was severed close to the cord and its distal stump divided until a strand containing a single active axon to the soleus nerve was found. The strand was placed across bipolar platinum recording electrodes. A bipolar platinum stimulating electrode was placed on the soleus nerve and a

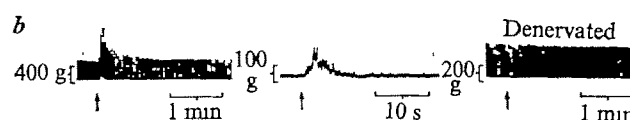
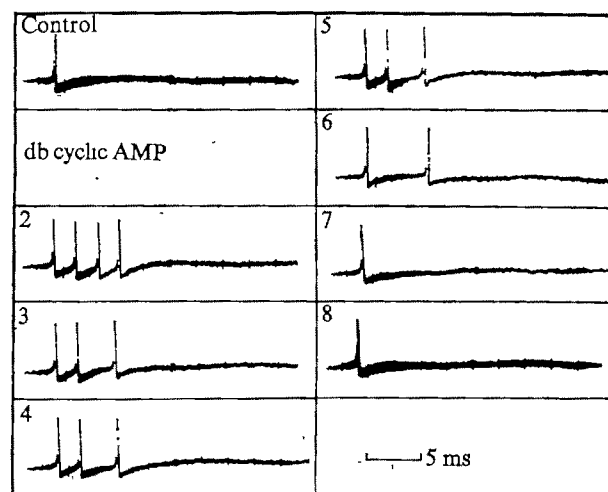


Fig. 1 *a*, SBR produced in a soleus motor axon by db cyclic AMP. The first trace shows the response to a single stimulus applied before the drug. Subsequent traces show the responses to similar stimuli applied once every 2.5 s after the administration of db cyclic AMP (100 µg kg⁻¹). The time mark represents 5 ms. *b*, Effects of db cyclic AMP on the force of contraction of the soleus muscle. The left record represents the response produced by administration of the agent (arrow, 100 µg kg⁻¹ intra-arterial) to a neurally stimulated muscle. The middle record represents the response to db cyclic AMP (arrow, 200 µg kg⁻¹ intra-arterial) of an unstimulated muscle. The right record represents the effect of the agent (arrow, 200 µg kg⁻¹ intra-arterial) on a chronically denervated muscle.

supramaximal pulse lasting 0.1 ms was applied every 2.5 s.

Denervated muscles were prepared by anaesthetising cats with intramuscular ketamine (25 mg kg⁻¹) and aseptically cutting the sciatic nerve at the sciatic notch. Two weeks later the animal was anaesthetised with intravenous chloralose (70 mg kg⁻¹) and the leg was prepared and mounted as before. A pair of electrodes was formed by sewing 33-gauge stainless steel wire in two series of concentric loops at the tendonous end of the muscle. A supra-maximal pulse lasting 2 ms was applied every 2.5 s.

Seven reagents were used. The first three, *N*²-*O*-dibutyl-adenosine-3',5'-monophosphoric acid, cyclic (db cyclic AMP), *N*²-*O*-dibutyl-guanosine-3',5'-monophosphoric acid, cyclic (db cyclic GMP) and adenosine-3',5'-monophosphoric acid, cyclic (cyclic AMP) are nucleotide derivatives. Of these, the first two cross cell membranes easily, while the last does not. The fourth, NaF, is an activator of adenylate cyclase⁷, the fifth, theophylline, is an inhibitor of phosphodiesterase and the last two, sodium butyrate and 5' adenosine monophosphate (5'AMP), are hydrolysis products of db cyclic AMP. Each compound was dissolved in 0.85% NaCl solution so that 0.1 ml contained the amount to be administered per kg, and 0.1 ml per kg of the solution was injected into the popliteal artery.

Dibutyl cyclic AMP in doses ranging from 50 to 200 µg kg⁻¹ caused SBR in motor axons (Fig. 1), with maximum intensity occurring after the first stimulus after the injection—less than 2.5 s after injection. Repetitive potentials were of high frequency, 300-400 Hz, but the train after each stimulus was always less than 12 ms. Neural activity was transmitted to the muscle where it caused repetitive activation and a brief tetanic contraction stronger than the simple twitch of the control period. This

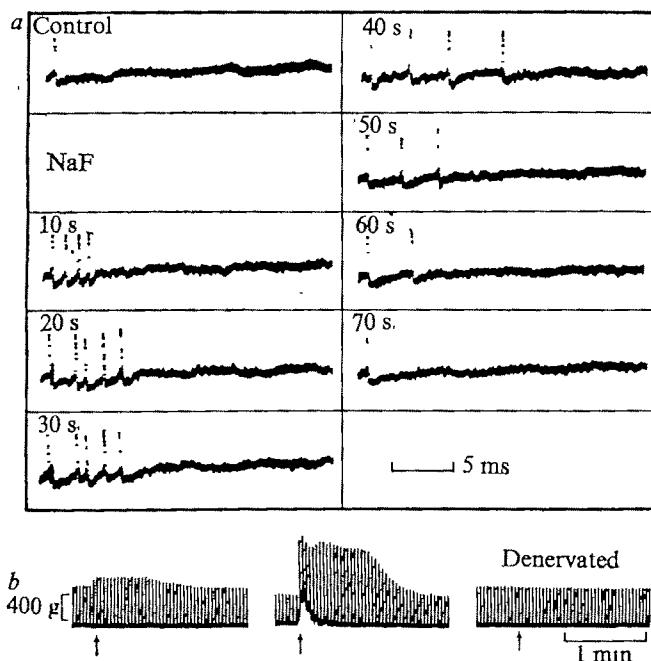


Fig. 2 *a*, SBR produced in a soleus motor axon by NaF. The first trace shows the response to a single stimulus applied before the drug. Subsequent traces show the responses to similar stimuli applied at the given time intervals after the intra-arterial administration of NaF ($200 \mu\text{g kg}^{-1}$). Time mark represents 5 ms. *b*, Effects of intra-arterial NaF (arrows) on the force of contraction of the soleus muscle. The first two records represent the effects of 50 and $200 \mu\text{g kg}^{-1}$, respectively, on a neurally stimulated preparation. The third trace represents the effect of $400 \mu\text{g kg}^{-1}$ on a chronically denervated muscle.

is shown in the mechanograms of Fig. 1, which also shows that the intensity, duration and time course of the repetitive activity were related to the dose of db cyclic AMP. Larger doses of db cyclic AMP ($> 100 \mu\text{g kg}^{-1}$) also initiated activity in unstimulated preparations. Immediately after injection, bursts of action potentials appeared in the nerve and the muscle underwent a series of rapid asynchronous contractions, causing the baseline of the record to rise (Fig. 1). The nerve activity consisted of brief bursts of between one and 150 action potentials which had a frequency of 300–500 pulses per s. Modally, the drug-initiated activity (DIA) consisted of 10–25 potentials and was complete within 3 s of injection. Dibutyl cyclic AMP in doses up to $800 \mu\text{g kg}^{-1}$ had no effect on denervated muscle (Fig. 1).

Cyclic AMP in doses up to $800 \mu\text{g kg}^{-1}$ or sodium butyrate or 5'AMP in doses up to 1 mg kg^{-1} had no effect on innervated or denervated preparations. Dibutyl cyclic GMP in doses of $1\text{--}2,000 \mu\text{g kg}^{-1}$ had no effect on innervated or denervated preparations and when given before or after the injection of db cyclic AMP, it did not affect the response to the latter nucleotide.

NaF produced responses which were qualitatively the same as those of db cyclic AMP (Fig. 2), causing SBR and DIA in motor axons, and potentiating the strength of indirectly evoked muscle contractions and causing asynchronous muscle contractions in unstimulated preparations. The threshold dose was $50 \mu\text{g kg}^{-1}$ and a ceiling of effect occurred at $400 \mu\text{g kg}^{-1}$. Injections of NaF up to $800 \mu\text{g kg}^{-1}$ had no effect on denervated muscle.

Theophylline caused SBR in motor axons and potentiation of muscle contraction strength (Fig. 3). The threshold dose was about $100 \mu\text{g kg}^{-1}$ and maximal effect occurred with doses of about $600 \mu\text{g kg}^{-1}$. The effect did not occur as rapidly as after db cyclic AMP or NaF; in most cases

5–10 s elapsed. The repetitive potentials were low in frequency, below 300 Hz, and the trains lasted up to 25 ms (Fig. 3).

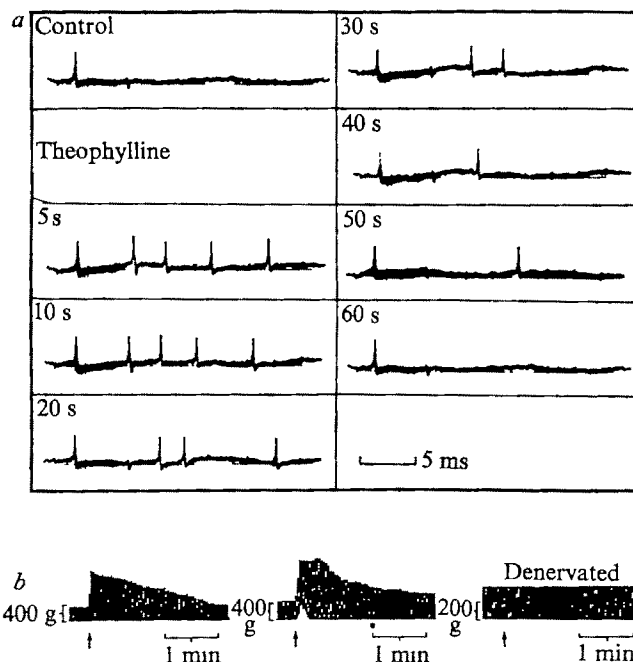
Theophylline also produced DIA in nerves and muscles but the dose required was larger than that needed to produce SBR. The activity differed from that initiated by db cyclic AMP and NaF in that there was a delay of several seconds between the injection of the drug and the appearance of the first potentials, and DIA was absent or much less intense if the stimulator was turned off for several minutes before injection. Theophylline had no effect on chronically denervated muscle.

Pretreatment with doses of theophylline below threshold for overt effects (such as $50 \mu\text{g kg}^{-1}$) lowered the threshold dose of db cyclic AMP or NaF by a factor of about 5. Theophylline also greatly increased the intensity and duration of all effects of db cyclic AMP or NaF.

There are several possible mechanisms responsible for SBR in motor axons but prolongation of the afternegativity of an action potential evoked in the nerve terminal is essential to them all^{1,9}. The prolonged afternegativity re-excites a faster recovering proximal segment of the axon (for example, the last node of Ranvier) and thereby produces SBR. Thus, in our experimental conditions, the production of SBR in the axon and/or potentiated contractions in its muscles shows that a reagent reacted with the nerve ending to prolong its afternegativity.

The lack of effect of each compound on chronically denervated muscle suggests that they do not cause a significant direct depolarisation of the post-junctional cell. This is substantiated by the reports that the compounds did not affect muscle membrane potential or amplitude of miniature end plate potentials (m.e.p.p.s) of nerve-muscle preparations. In the absence of significant

Fig. 3 *a*, SBR produced in a soleus motor axon by theophylline. The first trace shows the response to a single stimulus applied before the drug. Subsequent traces show the responses to similar stimuli applied at the given time intervals after intra-arterial administration of theophylline $100 \mu\text{g kg}^{-1}$. Time mark represents 5 ms. *b*, Effects of intra-arterial theophylline (arrows) on the force of contraction of the soleus muscle. The first two records represent the effects of 100 and $400 \mu\text{g kg}^{-1}$, respectively, on a neurally stimulated preparation. The third record represents the effect of $400 \mu\text{g kg}^{-1}$ on chronically denervated muscle.



postjunctional depolarisation, the most likely explanation for the production of DIA is that db cyclic AMP or NaF caused the nerve ending to become sufficiently depolarised to trigger action potentials in the axon and to release transmitter. Theophylline similarly seemed to cause depolarisation of nerve endings, but seemed to require prior activation of the nerve.

As cyclic AMP had no effect in our preparation and as its most significant difference from db cyclic AMP is in the capacity to penetrate cells, our results suggest that dibutyl cyclic AMP acts intracellularly to cause depolarisation of the nerve membrane. Dibutyl cyclic GMP, which also enters cells, also had no effect, suggesting that the effect is not a nonspecific response to intracellular nucleotides. Cyclic nucleotides have been shown to facilitate the influx of Na and/or Ca into cells¹⁰⁻¹⁴, and this can carry depolarising currents and trigger the release of transmitter¹⁵. These observations thus suggest that an increase in the influx of Na and/or Ca could have been the basis for the effects of dibutyl cyclic AMP in our experiments.

The similarity of the effects of NaF, an activator of adenylate cyclase, and dibutyl cyclic AMP, suggests that this enzyme, an important component of cyclic nucleotide systems, is present in motor nerve endings.

Because theophylline induces SBR in motor axons and potentiates and prolongs the effects of db cyclic AMP, phosphodiesterase is likely to be present in the nerve ending. The potentiation and prolongation of the effects of NaF by theophylline suggests that phosphodiesterase participates in the destruction of cyclic nucleotide formed after activation of adenylate cyclase in nerve endings.

If a cyclic nucleotide system was a link between the nerve action potential and transmitter release, it should have four characteristics. First, it should be quiescent until it is activated. Second, it should normally be activated by a nerve action potential. Third, it should be turned on, run its course and be turned off in a few ms. Fourth, it should be modified by exogenous cyclic nucleotides and reagents that affect the enzymes of the cyclic nucleotide system. Our results are in accord with these expectations and suggest mechanisms by which cyclic AMP might participate in neuromuscular transmission.

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Release and metabolism of substance P in rat hypothalamus

THE isolation and characterisation of the undecapeptide substance P from extracts of central nervous tissue has promoted renewed interest in the function of this compound in the central nervous system (CNS)¹. In addition, the development of a sensitive and specific radioimmunoassay² and complementary immunohistochemical techniques³ has provided evidence for a differential distribution of substance P in the CNS^{4,5}. Application of substance P to the spinal cord of neonatal rats has been shown to produce a potent depolarising action on motoneurons⁶. Elsewhere in the CNS iontophoretic application of substance P elicits a characteristic neuronal excitation which although slow in onset is long lasting⁷⁻¹⁰. If substance P is to be considered as a neurotransmitter in the CNS, demonstration of its release from nerve terminals in response to physiological depolarisation is the next essential prerequisite¹¹. We have therefore studied the release of substance P from rat hypothalamus, and have

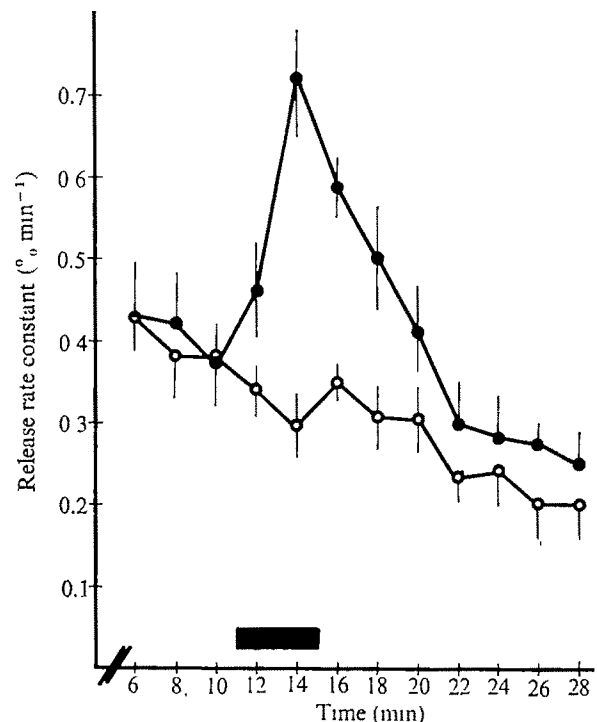


Fig. 1 Release of substance P-like immunoreactivity from rat hypothalamus slices. Adult male Wistar rats (200-250 g) were killed by decapitation, the brains were removed immediately and the hypothalamus was dissected using a modification of the method of Glowinski and Iversen²² (mean wet weight: 45.5 ± 2.1 mg; $n = 10$). Two hypothalami were used in each experiment, chopped in two directions at 0.2-mm intervals and transferred to a Perspex perfusion chamber. The slices were then superfused at 37 °C with Krebs-bicarbonate solution with glucose and containing 0.5% bovine serum albumin at a rate of 200 μ l min⁻¹. The superfusate was collected at 2-min intervals and stored on ice. Twelve minutes after the onset of perfusion, the potassium concentration of the medium was raised to 47 mM for 4 min (horizontal bar). In some experiments, the calcium concentration of the superfusion medium was reduced to 0.1 mM and the magnesium concentration raised to 3.5 mM. Samples of 200 μ l of serial superfusate fractions were removed for determination of substance P-like immunoreactivity by radioimmunoassay. Hypothalamic slices recovered after the superfusion were extracted and substance P-like immunoreactivity is expressed as a fractional rate constant, which represents the amount released per minute in each sample as a percentage of the total tissue content of peptide. Each point is the mean \pm s.e.m. for five experiments. ● Normal medium; ○, low Ca²⁺ medium.

demonstrated a potassium-evoked and calcium-dependent release of immunoreactive material. In addition preliminary results suggest that metabolic degradation rather than tissue uptake is responsible for the inactivation of substance P after its release from brain tissue.

Throughout this study substance P was measured by a radioimmunoassay, essentially similar to published methods². The lower limit of sensitivity was approximately 10 pg of substance P per assay sample. Antibody specificity was checked against the related peptide eleodoisin (Sigma), which failed to cross react with substance P even when present in 1,000-fold molar excess.

Using this radioimmunoassay procedure, we have found that rat hypothalamus contains a relatively high concentration of substance P-like immunoreactivity (SPLI)¹², in agreement with previous results in human brain¹. Rat hypothalamus was, therefore, chosen as a suitable tissue for release studies². A selective, calcium-dependent release of neurotransmitter candidates both *in vivo* and *in vitro* can be induced by raising the potassium concentration in the release studies². A selective, calcium-dependent release of neurotransmitter candidates both *in vivo* and *in vitro* can be induced by raising the potassium concentration in the external medium^{13,14}. Accordingly, to study the release of SPLI, rat hypothalamic slices were continuously superfused with Krebs-bicarbonate solution as described previously¹⁵, and potassium-evoked release was monitored. In

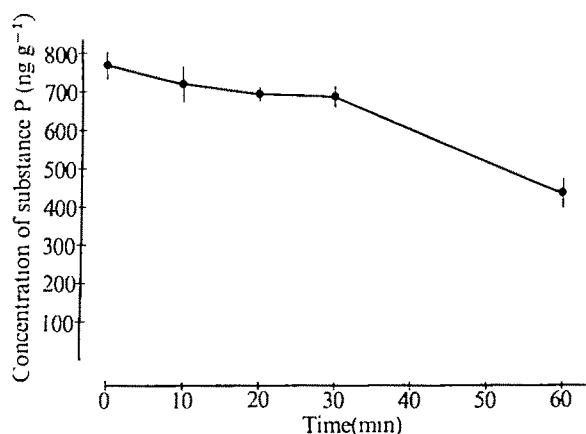


Fig. 2 Metabolism of substance P in rat hypothalamic slices. Portions (10 mg) of a suspension of rat hypothalamic slices were incubated in 1 ml of Krebs-bicarbonate solution with added glucose containing 0.5% bovine serum albumin at 37 °C for various times. At the end of incubation, samples were stored on ice and centrifuged at 1,500g for 10 min, and both supernatant and tissue were assayed for substance P-like immunoreactivity. Each point is the mean \pm s.e.m. for at least six determinations.

preliminary experiments the recovery of internal standards of substance P added to tissue extracts or superfusate samples was more than 94%. To prevent absorption of substance P to the apparatus and collection tubes, bovine serum albumin (0.5% w/v) was routinely added to the superfusion medium. Elevation of the potassium concentration in the superfusing medium to 47 mM for 4 min resulted in a marked increase in the rate of release of SPLI (Fig. 1). The maximum increase in release occurred within 4 min of potassium application, and efflux returned to spontaneous levels 6 min after restoration of normal potassium concentrations. Superfusion of hypothalamic slices with a medium in which the calcium concentration had been reduced to 0.1 mM and the magnesium concentration raised to 3.5 mM did not significantly affect the spontaneous efflux.

The marked increase in release evoked by 47 mM potassium, however, was completely abolished in the low Ca^{2+} and high Mg^{2+} medium. Potassium-evoked release of SPLI from rat hypothalamic tissue therefore seems to

be highly calcium dependent, as is the case with other neurotransmitter release mechanisms. SPLI remaining in the tissue at the end of each release experiment was determined. After 30 min of superfusion with normal Ca^{2+} medium, including 4 min of potassium stimulation, hypothalamic slices contained $663 \pm 50 \text{ ng g}^{-1}$ (mean \pm s.e., $n = 5$), a value not significantly different from that in freshly prepared slices not subjected to superfusion ($745 \pm 40 \text{ ng g}^{-1}$; $n = 6$) or in slices superfused with the low Ca^{2+} /high Mg^{2+} medium for 30 min ($637 \pm 86 \text{ ng g}^{-1}$; $n = 5$). The mean rate of spontaneous efflux of SPLI in normal or modified medium was equivalent to a total release of 8–10% of the tissue content during 30 min. The apparently small pool of tissue substance P available for release, and the high degree of retention of the peptide in the tissue, are similar to the results obtained with other transmitter substances in this type of release experiment^{14,15}.

If substance P is released as a neurotransmitter, mechanisms for inactivating the free peptide might be expected to exist in nervous tissue. To examine this possibility, we incubated hypothalamic slices in Krebs-bicarbonate medium at 37 °C for various times up to 60 min, without superfusion. Figure 2 shows that the rate of decline of SPLI in the hypothalamic tissue was slow: levels after 30 min were 89% of those at the onset of incubation. This figure is in good agreement with the values found in release experiments. In the absence of superfusion, however, no free peptide was detectable in the incubation medium at any time. After 60 min of incubation it was still not possible to detect SPLI in the incubation medium, although at this time tissue levels had fallen to 57% of the original values. It seems possible therefore that although tissue stores of substance P are reasonably stable, liberated substance P is susceptible to degradation within the hypothalamic tissue. Incubation of synthetic substance P with rat brain homogenates has been shown to result in rapid inactivation, with the release of all constituent amino acids¹⁶. In the release studies reported above, the continuous superfusion procedure presumably reduced such inactivation.

The possibility that the released peptide might be inactivated by a tissue uptake mechanism was also investigated in the rat cerebral cortex, dorsal and ventral spinal cord, substantia nigra, hypothalamus and cerebellum. Slices of these tissues (0.2 mm \times 0.2 mm) were incubated with ^{125}I -N¹-tyrosyl-substance P in the concentration range 10^{-7} – 10^{-6} M at 0, 25 and 37 °C. In no case did tissue: medium ratios significantly exceed unity, even with incubations of up to 40 min duration. Incubation of rat hypothalamic slices with unlabelled synthetic substance P at the same concentrations also failed to demonstrate any accumulation of the peptide. It seems unlikely, therefore, that substance P is actively accumulated by the tissue from which it is released.

Our results demonstrate that endogenous SPLI can be released from rat hypothalamic tissue in response to a depolarising stimulus by a calcium-dependent mechanism. Although the cellular origin of the released peptide is not known, it seems likely that release occurs from substance P-containing nerve terminals in the hypothalamus. Previous biochemical studies have shown that most of the substance P in homogenates of brain¹⁷ and hypothalamus¹⁸ is localised in synaptosome particles. Furthermore, immunohistochemical findings suggest that most of the SPLI in rat hypothalamus is concentrated in nerve terminals⁴. Our results also agree with those of earlier studies which showed the presence of substance P-like activity in the superfusates collected from spinal cord¹⁹ and cerebral cortex²⁰, although these studies were performed before the advent of the specific and sensitive radioimmunoassay procedure. There

have also been preliminary reports of a stimulus-evoked release of SPLI from rat spinal cord^{11,21}.

The above demonstration of release adds further weight to the hypothesis that substance P serves a neurotransmitter role in the nervous system. Although more information is needed on possible mechanisms of inactivation, our results suggest that tissue metabolism rather than a re-uptake mechanism is the important pathway for terminating the actions of the released peptide.

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Release of substance P-like immunoreactivity from isolated spinal cord of newborn rat

EVIDENCE is accumulating that substance P (H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH₂)¹ is an excitatory transmitter released from primary afferent nerve terminals in mammalian spinal cord (for reviews see refs 2 and 3). An important criterion for identification of a transmitter is the demonstration of release of the candidate substance from the nerve terminals in response to presynaptic stimulation. We report here the release of substance P-like immunoreactivity from isolated rat spinal cord into the bathing fluid during stimulation of dorsal roots.

Spinal cords from the thoracic level to the caudal end of 1-2-d-old Wistar rats were isolated, hemisected sagittally and placed in a 0.3-ml bath⁴. The preparation was perfused with oxygenated Krebs solution at 27 °C to which 6 µM dithiothreitol

was added to protect substance P against oxidation. Dithiothreitol at this concentration did not affect spinal reflexes, which indicated that the drug did not interfere with the oxygenation of the spinal cord. In most experiments, either 7 nM pepstatin⁵ or trasylol (0.5 U ml⁻¹) was added to the perfusion fluid to inhibit the degradation of substance P by proteases. Four to six lumbar dorsal roots were placed in suction electrodes for electrical stimulation. One of the ventral roots (L3-L5) was placed in another suction electrode with a tip diameter just fitting the size of the ventral root for extracellular recording, and the reflex responses were monitored during the stimulation periods. The perfusion rate was 0.2-0.6 ml min⁻¹, and after the preparation had been washed for at least 1 h, the perfusate was collected for 15 or 30 min into test tubes which each contained 1 ml of 60 mM dithiothreitol. Samples were frozen immediately, lyophilised and submitted to radioimmunoassay for substance P (ref. 6). The details of the assay system are described elsewhere⁷. Antisera against substance P were raised in rabbits, using synthetic substance P coupled with human α-globulin. ¹²⁵I-N¹-tyrosyl-substance P was used as tracer. The minimum detectable amount of substance P by the assay system was 2.5-5 pg per incubate. The antibody cross reacted with some of the shorter C-terminal analogues of substance P (deca-, nona- and octapeptides) but not with other shorter C-terminal analogues.

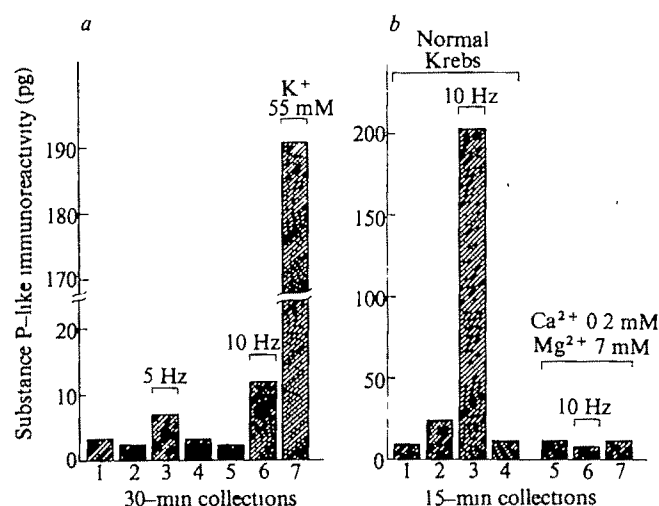


Fig. 1 Substance P-like immunoreactivity in the perfusates of isolated spinal cords of newborn rats. Each column represents the immunoreactivity expressed as an amount of substance P in a sample of about 7 ml. Stimulation periods and frequencies are indicated in the figure. The duration of each stimulating pulse was 0.01 ms in (a), and 0.3 ms in (b). In the experiment shown in (b), the preparation was first perfused with normal Krebs solution and then with a Ca²⁺-deficient and Mg²⁺-rich Krebs solution.

Figure 1 shows the results of two typical experiments. In the experiment shown in Fig. 1a, rectangular pulses of 0.01 ms were used for stimulation. During the resting periods, the amounts of substance P-like immunoreactivity in 30-min collection samples were below the limit of accurate measurement. Stimulation of the dorsal roots at a frequency of 5 Hz induced a slight increase, and stimulation at 10 Hz caused a definite increase in the release of substance P-like immunoreactivity. When the preparation was perfused with modified Krebs solution in which K⁺ concentration was increased to 55 mM, a large amount of the immunoreactivity was released into the perfusate. In the experiment illustrated in Fig. 1b, longer stimulation pulses (0.3 ms) were used to activate the smaller fibres in the dorsal roots. During the first stimulation period in normal Krebs solution a remarkable increase of substance P-like immunoreactivity in the perfusate was found, and this was associated with continual reflex responses throughout the stimulation period. But when the preparation was perfused with

modified Krebs solution containing 0.2 mM Ca^{2+} and 7 mM Mg^{2+} , which abolished monosynaptic and polysynaptic reflexes, the same stimulation produced no change in the immunoreactivity in the perfusate. Similar results were obtained when the experimental procedures were reversed; that is another spinal cord was first perfused with the Ca^{2+} -deficient and Mg^{2+} -rich Krebs solution, where the stimulation at 10 Hz for 15 min produced no reflex responses and no significant change of the immunoreactivity in the perfusate; the preparation was then perfused with normal Krebs solution and the same stimulation produced continual reflex responses and a marked increase in substance P-like immunoreactivity.

The results presented here demonstrate the calcium-dependent release of substance P-like immunoreactivity from the isolated rat spinal cord in response to stimulation of dorsal roots. Angelucci⁸ has shown that a substance P-like agent is released from perfused frog's spinal cord, but obtained no clear evidence as to whether its rate of release increased during reflex activity. Jessell *et al.*⁹ demonstrated the potassium-evoked and calcium-dependent release of substance P-like immunoreactivity from rat hypothalamus. The substance P-like immunoreactivity collected in our experiments is likely to have originated from nerve terminals of primary afferent fibres in the spinal cord, because previous studies^{10,11} gave good evidence for the occurrence of substance P in these fibres. Thus our findings support the view that substance P is an excitatory transmitter released from dorsal root fibres.

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Macromolecules, steroid binding and testosterone secretion by rabbit testes

THE formation of testosterone by the testis has been studied extensively and the general characteristics of the process have been described¹. But the mechanism by which testosterone escapes from the steroidogenic cell and then from the testis is largely unknown². Eik-Nes³ speculated that testosterone secretion could be explained by the presence of plasma constituents which bind the hormone. These plasma constituents might include erythrocytes, albumin and sex-hormone-binding globulin which have association constants for testosterone of 1×10^3 (ref. 4), 3×10^4 (ref. 5) and 1×10^5 (ref. 6), respectively. On the basis of this information, we hypothesised that testosterone secretion is dependent on diffusion from the steroidogenic cell into the blood and that this diffusion is enhanced by the presence of steroid-binding macromolecules in the blood. We report here a test of this hypothesis.

Rabbit testes were perfused *in vitro*⁷⁻⁹ at 10 ml per g of testis per h with medium containing macromolecules

which differed widely in steroid-binding capacity. These initial studies focused on serum albumin for three reasons: (1) its physical, chemical, and steroid-binding characteristics are well known; (2) it is the only steroid-binding protein commercially available in a highly purified but stable form, and (3) its plasma concentration and high capacity for steroids make it a likely candidate for one component of a macromolecular steroid sink in blood.

The medium for control perfusions consisted of Krebs-Ringer bicarbonate buffer, pH 7.4, 3% (w/v) bovine serum albumin (BSA), fraction V (Reheis Chemical Co.), washed bovine erythrocytes (25% in the medium), crystalline penicillin ($1,000 \text{ IU ml}^{-1}$), glucose (100 mg%) and a saturating concentration of NIH-LH-S17, ovine (100 ng ml^{-1}). The medium was gassed with a mixture of air and 5% carbon dioxide. For experimental perfusions, fraction V BSA was omitted and in some cases replaced with 3% 4 \times crystalline BSA (ICN Pharmaceuticals), 3% ovalbumin (Sigma) or 3% Dextran T70 (Pharmacia). Venous effluent from perfused testes was collected at hourly intervals during each experiment. The blood cells were sedimented by centrifugation and washed with an equal volume of 0.9% (w/v) saline. The saline was combined with the plasma portion of the venous effluent and frozen. The testosterone content of this material or of testicular homogenate was measured by gas-liquid chromatography¹⁰.

In vitro binding of testosterone to BSA, ovalbumin and Dextran was determined by equilibrium dialysis, according to Westphal's method¹¹. After dialysis for 18 h at 4 °C, testosterone ($0.5 \mu\text{g ml}^{-1}$) was found to be 86% bound to BSA, whereas it was only 22% bound to ovalbumin and less than 5% bound to Dextran.

In a further series of experiments, one testis from each of 24 rabbits was perfused with medium containing 3% fraction V BSA. The contralateral testes from six of the rabbits were perfused with medium without albumin; six with medium containing 4 \times crystalline albumin; six with medium containing 3% Dextran, and six with medium containing 3% ovalbumin. This experimental design enabled us to compare testosterone secretion by paired testes, thus eliminating the large variance in secretion between animals. Testosterone content of the venous effluent collected during the second hour of a 2-h perfusion was determined as described above.

The 24 testes perfused with medium containing fraction V secreted 17 ± 3.0 (s.e.m.) μg of testosterone per h. The results in Table 1 show that testosterone secretion by testes perfused with medium containing 4 \times crystalline BSA was similar to that obtained by paired testes receiving medium containing fraction V BSA. In contrast, testosterone secretion by testes perfused with medium either devoid of macromolecules or containing Dextran or ovalbumin, was only a quarter of that from testes receiving medium containing fraction V (Table 1).

Table 1 Effect of macromolecular constituents of perfusion medium on testosterone secretion by rabbit testes minus epididymides perfused *in vitro*

Macromolecule	% of Control*
4 \times recrystallised BSA	90 \pm 8.1†
None	26 \pm 3.5
Dextran T70	25 \pm 4.2
Ovalbumin	26 \pm 9.0

Testes were perfused for 2 h.

*Testosterone secretion was determined by measuring the testosterone content of testicular venous effluent collected during the second hour of perfusion and is expressed as a percentage of paired testis control perfused with medium containing fraction V BSA. Each value represents results from six rabbits.

†Standard error of the mean.

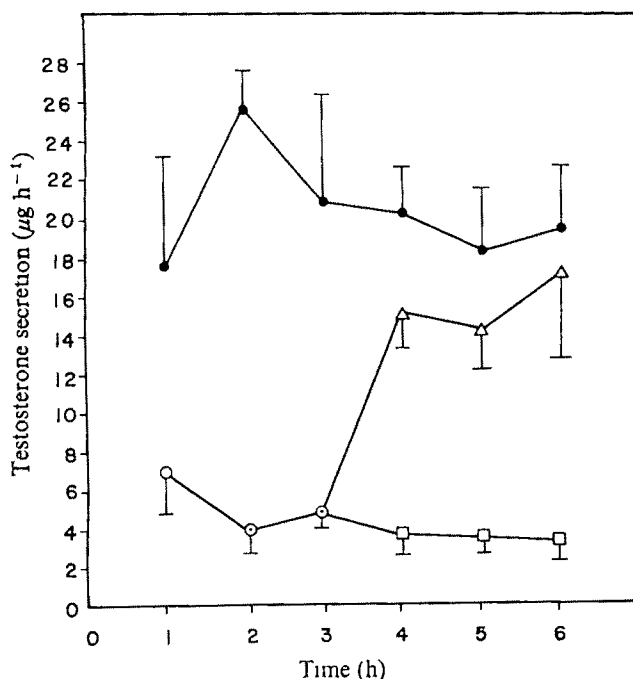


Fig. 1 Testosterone secretion by rabbit testes minus epididymides perfused *in vitro* with medium containing either 3% (w/v) fraction V BSA or 3% (w/v) Dextran. ●, Ten testes perfused with medium containing fraction V BSA for 6 h; ○, twenty testes perfused with the medium containing Dextran for the first, second and third hours of a 6-h perfusion experiment; △, ten testes switched to medium containing fraction V BSA during the fourth, fifth and sixth hours of perfusion. The latter ten testes were from the same rabbits that furnished the contralateral testes simultaneously perfused with medium containing fraction V BSA for 6 h. □, ten testes not switched but perfused with medium containing Dextran for 6 h. The \pm above or below each point represents the standard error of the mean.

Substitution of Dextran or ovalbumin for BSA maintains the plasma osmotic pressure, preventing tissue swelling during perfusion. Thus it is unlikely that the diminution in testosterone secretion in the absence of BSA was due to some nonspecific effect on tissue viability. The high rate of secretion by testes perfused with medium containing fraction V BSA was not explained by the presence of a macromolecular contaminant in fraction V BSA since the $4\times$ crystalline BSA, which also supported a high rate of testosterone secretion, gave a single band on gel electrophoresis.

The unlikely possibility that the lipid present in BSA but absent from Dextran served as a substrate for steroidogenesis was eliminated by showing that testosterone secretion was similar in eight testes perfused with medium containing Dextran ($2.9\pm 0.3\ \mu\text{g}$ of testosterone per h), and in eight testes perfused with the same medium plus the residue of a chloroform-methanol extract of an equivalent amount of medium containing 3% fraction V BSA ($2.9\pm 0.4\ \mu\text{g}$ of testosterone per h).

Because these experiments were of short duration, the results could not eliminate the possibility that diminished testosterone secretion in the absence of albumin was only a transient phenomenon, or that reduced testosterone secretion in the absence of albumin resulted from some irreversible change in the steroidogenic cells. We therefore perfused one testis from each of 10 rabbits for 6 h with medium containing 3% fraction V BSA. The contralateral testis from each rabbit was perfused simultaneously with medium containing 3% Dextran for 3 h; then with medium containing 3% fraction V for a further 3 h. Finally, one testis from each of 10 more rabbits was perfused with medium containing Dextran for 6 h. Hourly samples of

venous effluent were collected and testosterone was quantified as described above. Figure 1 shows that testes perfused with medium containing fraction V BSA secreted $19\pm 3\ \mu\text{g}$ of testosterone per h in 6 h. In contrast, testes perfused with artificial medium containing Dextran secreted $4.5\pm 0.7\ \mu\text{g}$ of testosterone per h in 6 h. Importantly, the low testosterone secretion, obtained when testes were perfused with Dextran containing medium for 3 h, returned to the control rate of secretion after being changed to medium containing fraction V BSA (Figure 1). These results show that the low rate of secretion in the presence of Dextran did not increase spontaneously during 6 h of perfusion. Moreover, Dextran did not cause irreversible damage to the testicular steroidogenic cell within 3 h.

It can be calculated from the results in Fig. 1 that testes perfused with medium containing either BSA or Dextran produced $114\ \mu\text{g}$ of testosterone ($19\ \mu\text{g h}^{-1}$ for 6 h) or $27\ \mu\text{g}$ of testosterone ($4.5\ \mu\text{g h}^{-1}$ for 6 h), respectively, during 6 h of perfusion. The testosterone content of testes perfused with medium containing either BSA or Dextran for 6 h was $2.0\pm 0.3\ \mu\text{g}$ and $3.5\pm 0.7\ \mu\text{g}$ of testosterone per testis, respectively. This difference of $1.5\ \mu\text{g}$ in testicular content fails to account for the difference of $87\ \mu\text{g}$ in the amount of testosterone secreted by testes perfused with medium containing BSA, rather than with Dextran. Similarly, the testicular content of 5α androstan- 17β -ol-3-one, 5α androstan- $3\alpha,17\beta$ -diol, 5α androstan $3\beta,17\beta$ -diol and $\Delta 4$ androst- $3,17$ -dione failed to account for significant amounts of the $87\text{-}\mu\text{g}$ discrepancy in testosterone secretion by testes perfused with medium containing Dextran rather than fraction V BSA. Therefore, diminished testosterone secretion in the absence of BSA cannot be explained by a failure of testosterone to be released from the testis or by an intratesticular accumulation of testosterone or some common testosterone metabolites.

These are the first experimental results to confirm Westphal's¹ hypothesis that, "binding proteins facilitate the passage of the (predominantly hydrophobic) steroids through the endothelium of the capillary walls". Further experimentation is required: to determine the relative impact of testosterone binding to erythrocytes, albumin or (sex-hormone-binding) globulin on testosterone secretion; to determine the mechanism by which testosterone binding to blood plasma macromolecules increases testosterone secretion; and finally to determine the effect of experimental treatments which alter either the concentration or steroid binding characteristics of blood plasma macromolecules on testosterone secretion.

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Recognition of lysosomal glycosidases *in vivo* inhibited by modified glycoproteins

PURIFIED liver lysosomal glycosidases display very short plasma survival times following intravenous injection. The mechanism by which these hydrolases are cleared from plasma involves specific recognition of the enzymes by way of sites associated with the enzyme molecules. In our study, a modified plasma glycoprotein terminating in *N*-acetyl-glucosamine has been shown to inhibit recognition and clearance of several purified lysosomal glycosidases.

Lysosomal glycosidases are glycoproteins^{1,2} mainly intracellular³ with only trace amounts normally occurring in plasma and extracellular fluids. Recent work⁴⁻⁷ indicates that various highly purified lysosomal glycosidases are very rapidly cleared from the circulation after intravenous administration. Rapid plasma clearance of lysosomal glycosidases seems to be mediated by liver plasma membrane binding sites or receptors⁸ with subsequent endocytosis and incorporation into liver lysosomes⁷. Rapid clearance is not a characteristic of all forms of a given glycosidase since some species (for example, purified normal serum β -glucuronidase)⁷ are not cleared and display relatively long plasma half lives. Moreover, the administration of diisopropylfluorophosphate⁹ rapidly raises the concentration of slow clearance forms of β -glucuronidase in rat plasma.

Extracellular survival of many glycoproteins is determined by the exposed or terminal sugar residues associated with the carbohydrate chains⁹. Nearly all plasma glycoproteins, for instance, having galactose as the penultimate sugar are rapidly cleared from plasma when the terminal sugar (sialic acid) is removed¹⁰. The mechanism of clearance of various asialo-glycoproteins with exposed galactose residues involves binding to a liver parenchymal cell plasma membrane receptor¹¹. More recently, a second clearance route dependent on previous sequential removal of two sugars (sialic acid and galactose) from the carbohydrate chains of orosomucoid, has been described^{12,13}. Enzymatic removal of two sugars from orosomucoid results in the exposure of *N*-acetyl glucosamine. Plasma clearance of agalacto-orosomucoid seems to be mediated by recognition of terminal *N*-acetyl-glucosaminyl residues.

Several lines of evidence suggest that carbohydrate residues are important in mediating the clearance of lysosomal glycosidases¹⁴. In the hope of identifying the recognition site(s) associated with rapidly-cleared glycosidases, clearance competition experiments were undertaken using a family of purified rat liver lysosomal hydrolases including β -glucuronidase, β -galactosidase, *N*-acetyl- β -D-glucosaminidase and α -fucosidase. Asialo-orosomucoid (ASOR) and agalacto-orosomucoid (AGOR) were used as antagonists.

Clearance of various glycosidase activities was followed in the anaesthetised rat after a brief intravenous infusion of enzyme. The enzyme preparations were prepared from rat liver lysosomes and rat preputial gland. Rat preputial gland is an extremely rich source of β -glucuronidase from which large quantities of highly purified enzymes can be isolated. β -Glucuronidase from rat preputial gland and rat liver lysosomes are indistinguishable on the basis of catalytic properties⁷, molecular dimensions^{15,16} and clearance following intravenous infusion⁷.

Rat preputial gland β -glucuronidase, purified by the method of Ohtsuka and Wakabayashi¹⁷ had a specific activity of 2,000 phenolphthalein glucuronic acid (PGA) units mg^{-1} (see Table 1). Liver lysosomal β -glucuronidase, isolated by a modification of the method of Stahl and Touster¹⁸ using an affinity chromatography step described by Owens *et al.*¹⁹, had a specific activity of 1,000 PGA units mg^{-1} . β -Galactosidase was isolated from rat liver lysosomes with subsequent purification on concanavalin A-Sepharose as described by Stahl *et al.*⁷ and *p*-aminophenyl- β -D-thiogalactosyl-Sepharose as described by Distler and Jourdan¹⁹. The preparation used had a specific activity of 50 *p*-nitrophenyl (PNP) units mg^{-1} . α -Fucosidase, from rat liver lysosomes, was purified by affinity chromatography using ξ -amino-caproyl-fucosamine-Sepharose (Fucosylex, Miles). *N*-acetyl- β -D-glucosaminidase was isolated from rat liver lysosomes, as described by Stahl *et al.*⁷, to a specific activity of 880 PNP units mg^{-1} .

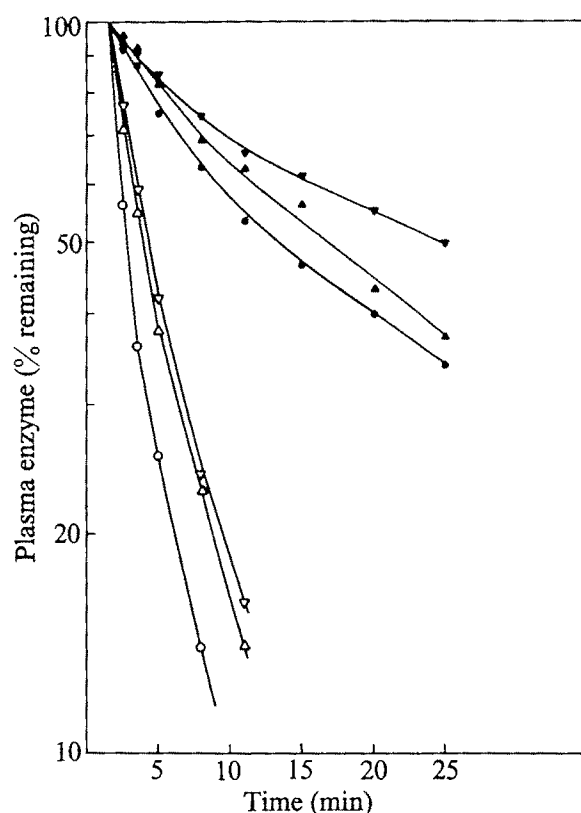


Fig. 1 Effect of AGOR on the clearance of liver lysosomal β -glucuronidase, β -galactosidase, and NAGA. Enzyme was infused as described in Table 1 with and without 1.0 mg AGOR. Plasma enzyme levels are expressed, after subtraction of pre-infusion plasma levels, as a percentage of the 1.5-min sample. \circ , β -Glucuronidase; Δ , β -galactosidase; ∇ , *N*-acetyl- β -glucosaminidase; \bullet , β -glucuronidase + AGOR; \blacktriangle , β -galactosidase + AGOR; \blacktriangledown , *N*-acetyl- β -glucosaminidase + AGOR.

All enzymes were administered in 0.15 M NaCl. Asialo-orosomucoid was prepared by digestion of purified orosomucoid with *Clostridium perfringens* neuraminidase. The latter was protease-free. Agalacto-orosomucoid was prepared from asialo-orosomucoid by treating the latter with β -galactosidase isolated from the culture fluid of *Diplococcus pneumoniae*.

Clearance of three liver lysosomal glycosidases, β -glucuronidase, β -galactosidase and *N*-acetyl- β -D-glucosaminidase is summarised in Fig. 1. Two other enzymes, rat preputial β -glucuronidase and liver lysosomal α -fucosidase were also studied (Table 1). Clearance profiles in Fig. 1, constructed from analysis of sequential arterial plasma samples, demonstrate the very rapid nature of enzyme

Table 1 Effect of agalacto-orosomucoid and asialo-orosomucoid on the clearance of various lysosomal hydrolases

Enzyme	Inhibitor	0 min	Plasma level (units ml ⁻¹)			Plasma half life
			1.5 min	5.0 min	30 min	
L- β -Glucuronidase	—	0.11	10.6	2.8	0.14	1.3
L- β -Glucuronidase	AGOR	0.33	14.3	10.7	3.9	11.8
—	AGOR	0.16	0.2	0.2	0.2	
P- β -Glucuronidase	—	0.07	4.1	2.7	0.29	4.0
P- β -Glucuronidase	ASOR	0.14	5.6	3.0	0.85	4.0
P- β -Glucuronidase	AGOR	0.14	6.7	6.3	3.5	> 30
NAGA	—	0.49	2.5	1.1	0.04	2.8
NAGA	ASOR	0.36	2.6	0.9	0.09	3.0
NAGA	AGOR	0.54	5.0	4.2	2.0	23.0
—	AGOR	0.51	0.5	0.6	0.8	
β -Galactosidase	—	0.17	1.2	0.47	< 0.01	2.5
β -Galactosidase	ASOR	0.14	1.2	0.63	0.14	4.0
β -Galactosidase	AGOR	0.09	1.5	1.3	0.44	15.0
—	AGOR	0.2	0.2	0.1	0.1	
α -Fucosidase	—	1.3	2.0	1.7	0.5	14.5
α -Fucosidase	ASOR	0.9	2.0	1.5	0.5	12.5
α -Fucosidase	AGOR	1.2	2.5	2.4	1.5	36.0
—	AGOR	0.7	0.6	0.6	0.8	

Lysosomal (L) β -glucuronidase, 20 PGA units (20 μ g), preputial gland (P) β -glucuronidase, 10 PGA units (5 μ g), β -galactosidase, 5 PNP units (100 μ g), *N*-acetyl- β -D-glucosaminidase, 20 PNP units (22 μ g); and α -L-fucosidase, 5 PNP units (11 μ g) were infused by femoral vein cannula into the anaesthetised rat without (—) or with ASOR (1 mg) or AGOR (1 mg), respectively, in a total volume of 0.5 ml. Female Wistar rats (50–60 g), anaesthetised with sodium pentobarbital (30 mg kg⁻¹, intraperitoneally), were used throughout. Femoral vein and carotid artery cannulations were made and enzyme preparations were infused for 70 s. The first blood sample was taken at 90 s using heparinised haematocrit tubes. Further details are described by Stahl *et al.*⁷. β -Glucuronidase was assayed using PGA¹⁶ or 4-methylumbelliferyl-(MU)-glucuronide as described by Stahl *et al.*⁷. β -Galactosidase was assayed using 2.5 mM PNP- β -D-galactoside or MU- β -D-galactoside. The reaction was run in 0.1 M Na acetate pH 4.3, containing 0.5 M NaCl and terminated by the addition of alkaline stopping reagent¹⁸. *N*-acetyl- β -D-glucosaminidase and α -fucosidase were assayed using the appropriate PNP- and MU-glycosides as described by Stahl *et al.*⁷. All units of enzymatic activity are expressed as μ mol product formed per h. Protein was determined by the Miller method²⁰. The results are the average of two animals per enzyme determination. Results for AGOR (1 mg) infusion are from one animal only. Plasma enzyme levels are expressed as MU units per ml plasma. Preinfusion control plasma enzyme levels have been subtracted from post-infusion values. Plasma half life was estimated as the time required to reach 50% of the 1.5-min plasma level. NAGA = *N*-acetyl- β -D-glucosaminidase.

clearance. The actual plasma enzyme levels at selected post-infusion time points appear in Table 1.

When various lysosomal glycosidases were infused with 1 mg AGOR, the enzyme clearance curves were shifted considerably to the right (Fig. 1). As expected, plasma half life for each enzyme was appreciably extended and plasma enzyme levels 30 min after infusion were enhanced many times (Table 2). Injection of 1 mg AGOR alone (Table 2) had no effect on plasma glycosidase levels. Corresponding experiments were undertaken to determine whether ASOR has any inhibitory effects on enzyme clearance. ASOR (1 mg) had no effect on clearance of the five lysosomal enzyme preparations (Table 2).

To test whether inhibition of clearance of β -glucuronidase is dependent on the concentration of inhibitor, a series of doses of AGOR were administered with a tracer dose (0.005 mg) of preputial β -glucuronidase. The rate of enzyme clearance was measured as described previously⁷. Briefly, for each dose of AGOR, a semi-log plot was made of plasma enzyme level against time. The initial enzyme clearance rate was estimated from the slope of a line drawn through the first few (2–4) time points. Clearance rates,

expressed as units ml⁻¹ min⁻¹, are summarised in Table 2 for a series of doses of AGOR. As little as 30 μ g of AGOR reduced β -glucuronidase clearance by 25%. The dose-dependent nature of AGOR inhibition of preputial β -glucuronidase clearance suggests that the two are competing for the same receptor site. However, this point will require more kinetic and structural analysis.

Previous work from our laboratory¹⁴ with two rat lysosomal glycosidases, β -glucuronidase and *N*-acetyl- β -D-glucosaminidase, indicated that periodate oxidation of the enzymes resulted in loss of rapid clearance without appreciable changes in catalytic activity. These results, along with those of Hickman *et al.*²¹, suggest that sugar residues are important in enzyme recognition and uptake by cells. The observations reported in the present paper where an *N*-acetyl-glucosaminyl terminal glycoprotein inhibited clearance of a family of lysosomal glycosidases support and extend earlier work implicating carbohydrate residues in the recognition site. Further, the results suggest that the recognition site includes *N*-acetyl glucosamine. Confirmation of this must await chemical analysis of glycopeptides derived from lysosomal enzymes. Of considerable interest are the recent findings of Achord, Brot, Gonzales-Noriega and Sly (in preparation), that highly purified human placental β -glucuronidase is rapidly cleared from the circulation in the rat and that the clearance system seems to recognise the carbohydrate moiety of the enzyme. Our results would suggest that the liver recognition system, described in mammals by Stockert *et al.*²² and in birds by Lunney and Ashwell¹³ and Regoeczi *et al.*²³ for modified glycoproteins, may function to maintain the very low levels of lysosomal enzymes found in extracellular fluids. The results may prove to be important in enzyme replacement therapy for lysosomal enzyme deficiency storage diseases.

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Table 2 Effect of agalacto-orosomucoid on β -glucuronidase clearance rate

Enzyme (units)	Inhibitor (mg)	β -Glucuronidase clearance rate (units ml ⁻¹ min ⁻¹)
β -Glucuronidase (10)	—	0.55
	AGOR (0.03)	0.32
	AGOR (0.1)	0.30
	AGOR (1.0)	0.21

The effect of AGOR on the initial plasma clearance rate of preputial β -glucuronidase (10 PGA units) was estimated by plotting plasma enzyme levels after termination of infusion, as described in Table 1, against time. The rate was calculated from the slope of a line connecting the first few (2–4) points on the clearance curve as described in ref. 7. Various amounts of AGOR were added to the infusate.

human β -glucuronidase before publication. We also thank Dr Gilbert Ashwell, for providing ASOR and AGOR and for helpful suggestions, and Dr Oscar Touster, Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee (Opheim and Touster, in preparation), for providing the method of purifying α -fucosidase.

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Model for the structure of the gastric mucous gel

THE surface of the stomach is covered by a layer of mucous gel which protects the underlying mucosa from the harmful, acidic stomach contents. The principal component of the gel has been isolated from pig gastric mucus, purified, and shown to be a glycoprotein of molecular weight 2×10^6 (Table 1)^{1,2}. This glycoprotein consists of four equal sized subunits (molecular weight 5×10^5) joined by disulphide bridges^{3,4}. Each glycoprotein subunit consists of a protein core, 14% by weight of the glycoprotein, with carbohydrate side chains attached. The protein core consists of two regions, one rich in serine, threonine and proline and bearing all the carbohydrate, the other having an amino acid composition characteristic of a globular protein. The latter contains cystine residues which bridge the four subunits. Mild reducing agents and proteolytic enzymes each split the glycoprotein into four subunits^{4,5}. The carbohydrate chains, approximately 15 residues in a branched structure, account for 82% by weight of the glycoprotein and carry ester sulphate residues. The problem therefore is to explain how such a glycoprotein molecule can associate to form a gel which *in vivo* is essentially impermeable to proteolytic enzymes and which can act as a barrier to protons probably by supporting a pH gradient.

The mucous gel isolated directly from the stomach is clearly viscoelastic, despite its non-uniform concentration. It can be completely solubilised by guanidinium chloride or by, homogenisation in water, demonstrating that the gel is stabilised solely by non-covalent interactions between the component glycoprotein molecules⁶. The solubilised gastric glycoprotein, in 0.2 M KCl, has a high intrinsic viscosity of 320 ml g⁻¹ but

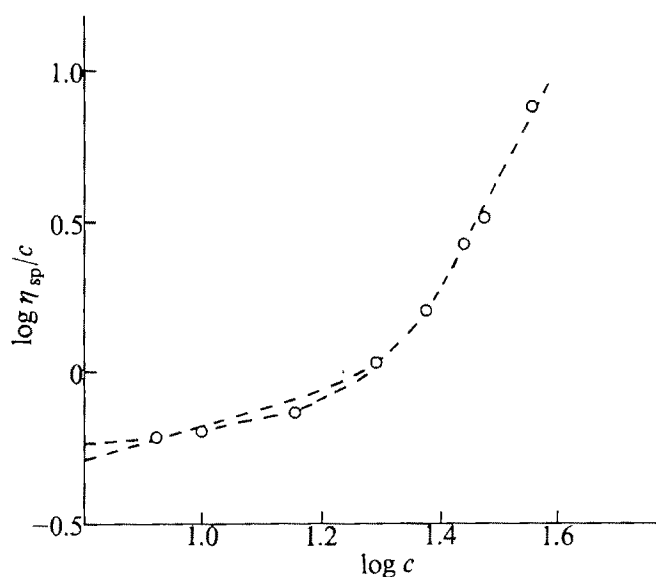


Fig. 1 The dependence of the viscosity of the gastric glycoprotein on concentration: plot of $\log \eta_{sp}/c$ against $\log c$. Viscosities were measured using a Couette viscometer. Solvent: 0.2 M KCl, 0.02% (w/v) azide; 0.02 M K acetate buffer, pH 5.5.

the concentration dependence of its viscous, sedimentation and diffusion properties indicate a surprisingly low degree of intermolecular interaction (see Table 1) (Fig. 1). At higher glycoprotein concentrations of 20–30 mg ml⁻¹ the viscosity indicates a deviation from this behaviour and intermolecular interaction assumes an increasingly significant contribution to the viscosity of the solution. This is demonstrated by the logarithmic plot of reduced specific viscosity against concentration (Fig. 1), where the increase in slope on going from low to higher concentrations of glycoprotein is characteristic of the behaviour of certain interacting synthetic polymers⁷. The major change in behaviour occurs in the concentration range 20–25 mg ml⁻¹. Concentrating the glycoprotein further will result in gel formation *in vitro*, although the nature of this gel and the exact conditions for its formation have yet to be studied in detail. The average concentration of the glycoprotein found in mucous gel isolated directly from the stomach is in the region of 30–40 mg ml⁻¹, that is, in the range in which intermolecular interaction is shown above to be occurring.

The flow properties of the glycoprotein at low concentrations in 0.2 M KCl solution can be accounted for by a molecule which is highly expanded and approximately spherical⁸. An indication of the solution volume occupied by the glycoprotein at high dilution is given by its effective hydrodynamic volume, V_e , from the relationship

$$V_e = (f/6\pi\eta_0)^3(4\pi\mathcal{N}/3M)$$

assuming the molecule is spherical. f is the frictional coefficient calculated from the diffusion coefficient, η_0 is the viscosity of the solvent, \mathcal{N} is Avogadro's number and M the molecular weight. For the glycoprotein in 0.2 M KCl, V_e has a value of 40 ml g⁻¹. V_e does not define the precise geometry of the molecule itself⁸ but it does give an indication of the volume within which the probability of intermolecular interaction becomes appreciable. On this basis, therefore, the sphere of interaction of glycoprotein molecules will spread over the total solution volume at a solute concentration of 25 mg ml⁻¹. This, it will be noted, is in the concentration range at which independent experimental data shows intermolecular interaction to become significant (Fig. 1). A large volume of solvent is thus enclosed by the glycoprotein molecule and it is quite possible therefore to account for the existence of solutions of concentration higher than 25 mg ml⁻¹ by an overlap of molecular volumes or interdigitation. This would, however, reduce the

Table 1 Properties of the glycoprotein from pig gastric mucus

Chemical composition ^a	(% by weight of freeze-dried glycoprotein)	
Carbohydrate 73.6	galactose (24.9), glucosamine (23.3), galactosamine (8.7), fucose (14.3), <i>N</i> -acetylneuraminic acid (2.4).	
Protein 12.9	Sulphate 3.1	Water 10.4 (by difference)
Physical data ^a	(in 0.2 M KCl; 0.02 M K acetate; 0.02% (w/v) Na azide; pH 5.5).	
$s_{20}^{0, w}$	18.7S	
$D_{20}^{0, w}$	$0.69 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$	
Molecular weight	2×10^6	
f	$5.96 \times 10^{-7} \text{ cm s}^{-1}$	
V_e	40 ml g ⁻¹	
Concentration dependence of physical parameters:		
Sedimentation coefficient	$K_s [= s^0 d(s^{-1})/dc]$	260 ml g ⁻¹
Diffusion coefficient	$K_D [= -(1/D^0) dD/dc]$	0 ml g ⁻¹
Viscosity (Huggin's constant)	$k [= (\eta_{sp}/c - [\eta])(1/[\eta]^2)c]$	0.3

Sugars were analysed by gas-liquid chromatography and the protein is a summation of the contents of the individual amino acids.

rotational relaxation of the molecule, lead to a rapid rise in viscosity and, at some point depending on the nature and strength of non-covalent intermolecular forces, to gel formation. This point is further supported by calculations of the maximum volume which could be occupied by the glycosylated part (< 90% by weight) of the molecule assuming fully extended sugar chains eight residues in length⁹ forming a cylinder on four extended glycosylated polypeptide chains each 565 residues long. This model leads to a volume of 12 ml g⁻¹ which is a substantial proportion of the effective hydrodynamic volume V_e . The shortfall between this value and that of 40 ml g⁻¹ found experimentally suggests that the four glycoprotein subunits may be flexible about the non-glycosylated disulphide bonded regions of the polypeptide chains.

Based on the above evidence we propose the following model for the structure of the gastric mucous gel. The gel is composed of highly expanded, solution-filling, glycoprotein molecules which, packed together, interact at or near their surfaces. A large proportion of the solvent is thus intramolecular and the polymer extends more or less evenly throughout the gel. This model contrasts with those structures elucidated for other polysaccharide gels such as agar¹⁰ where a much lower concentration of solute can form a relatively low density open network of molecular fibres. The gastric mucous gel, therefore, would be expected to be relatively impermeable to large molecules such as enzymes compared with the free diffusion of such through agar gels. Further, such a structure should reduce considerably any turbulence in the solvent within the gel allowing a relatively stable gradient of pH to be maintained at the surface of the stomach.

This model predicts that gel formation at a given concentration should be markedly dependent on the effective volume in solution of the glycoprotein molecule. Factors such as ionic strength which have been shown to change the size of V_e do in fact markedly affect the intermolecular interactions⁸. It is not yet clear what could stimulate intermolecular interaction between polar chemical groups which appear to be strongly solvated, although there is evidence that removal of water from such structures brings about preferential interaction between sugar chains¹⁰.

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Cloning of all *EcoRI* fragments from phage λ in *E. coli*

CLONING of DNA fragments is a new powerful technique in molecular genetics and a number of interesting experiments involving both prokaryotic and eukaryotic genomes have already been reported (see ref. 1 for review). We present here results concerning the cloning of all *EcoRI* (ref. 2) fragments from phage λ DNA in *Escherichia coli* using as a vector plasmid pSC101 (ref. 3). To achieve this, we had to solve the problem of propagating the λ fragment containing the immunity region since the phage strain used had a temperature-sensitive mutation in that region.

DNA from the lysogenising strain c1857 of phage λ and the supercoiled form of plasmid pSC101, were digested at 37°C with restriction enzyme *EcoRI* (prepared according to a modification of the method of Yoshimori⁴) in 100 mM Tris, pH 7.5, and 10 mM MgCl₂ (ref. 5). After digestion was complete, as judged by electrophoresis of the DNAs, *EcoRI* was inactivated by heating the incubation mixtures at 65°C for 5 min. The two digests were then mixed together, slowly chilled to 0°C to allow reannealing of the "sticky ends" formed by *EcoRI* to occur and incubated for 16 h at 0°C and 1 h at 14°C in 50 mM Tris, pH 7.5, 10 mM MgCl₂, 1 mM EDTA, 0.035 mM ATP, 10 mM dithiothreitol⁶ and polynucleotide ligase from T4-infected *E. coli* (from Miles or from Dr F. Rougeon). The ligated mixture was used to transform strain HB 101 (ref. 7) of *E. coli*. The transformants were plated on L agar⁸ containing 15 (or 10, ref. 9) $\mu\text{g ml}^{-1}$ tetracycline, grown overnight at 37°C and further purified on plates containing tetracycline. Selection of recombinant clones was carried out either by a group screening using gel electrophoresis, or by the hybridisation technique of Grunstein and Hogness¹¹.

In an experiment, in which 85 clones were screened in groups of 7-8, at least 10 were found to contain λ fragments 3, 4 or 5 (the numbering of the six *EcoRI* λ fragments is according to decreasing molecular weight¹¹);

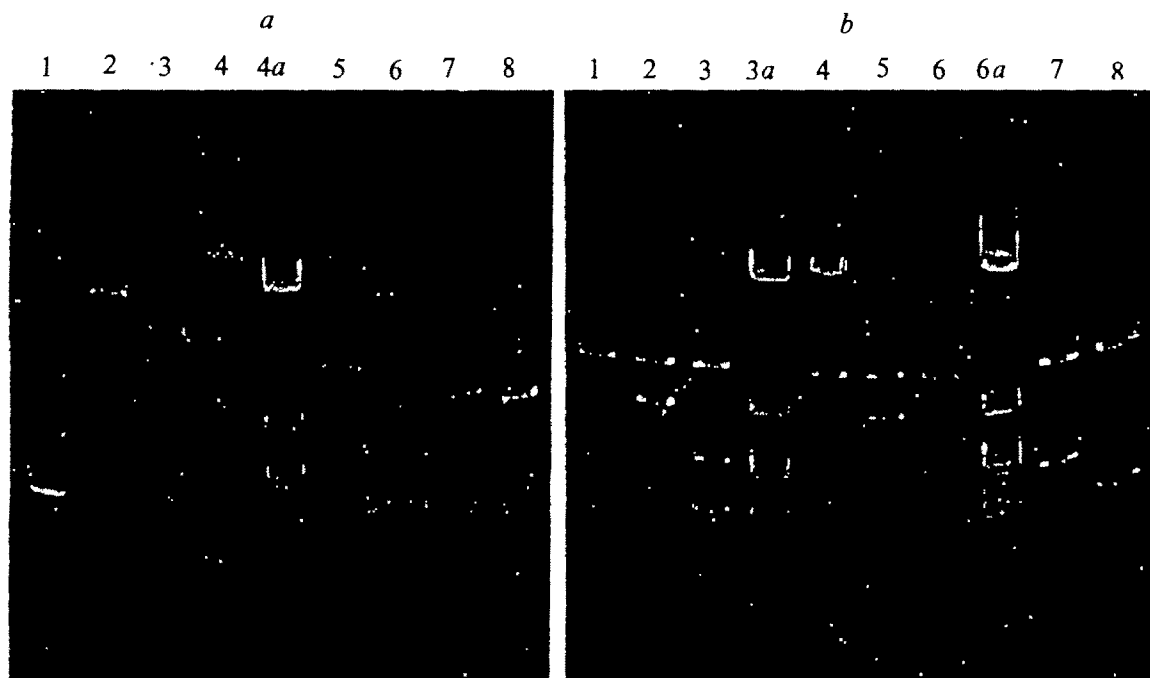


Fig. 1. Electrophoresis of recombinant pSC101- λ fragment plasmids before (a) and after (b) *EcoRI* digestion. a, Slots contain: 1, supercoiled circular pSC101; 2, pSC101 + fragment 2 (two copies); 3, pSC101 + fragments 3+5; 4, pSC101 + fragments 1+6; 4a, *EcoRI* fragments of λ DNA; 5, pSC101 + fragment 2; 6, pSC101 + fragment 3; 7, pSC101 + fragment 4; 8, pSC101 + fragment 5. b, Slots 1-8 contain the same plasmids as in a after digestion with *EcoRI*; slots 3a and 6a contain *EcoRI* digests of λ DNA; in slot 3a fragments were heated for 5 min at 70 °C to show the separation of fragments 1 and 6, which gives a single band in non-heated *EcoRI* fragments (slot 6a).

these range in molecular weight from 3.5 to 3×10^6 . One additional clone contained two λ fragments, 3 and 5, which are non-adjacent in the genome and account together for 6.5×10^6 of DNA. Still another clone contained fragments 1 and 6; these fragments are bound together through the natural cohesive ends of λ DNA; they contain the whole left arm of the genome and have a molecular weight of 16×10^6 ; obviously, these fragments cannot be inserted separately since each one of them lacks an *EcoRI* sticky end in the linear form of λ DNA. The easier integration of low molecular weight compared to high molecular weight fragments is expected; our failure to find recombinants harbouring λ fragment 2, which has a molecular weight of 4×10^6 , was, therefore, at first sight, surprising.

An experiment in which λ fragment 2 was eluted from Agarose¹², ligated with pSC101 and used for transfection, did not yield any recombinant plasmid, in spite of the fact that 600 colonies were examined by the hybridisation technique; in contrast, a parallel experiment with fragments 3 and 4 was successful; this suggests that our negative results with fragment 2 were not due to the fact that fragments eluted from Agarose cannot be ligated.

Since fragment 2 comprises the λ immunity region, which is the locus of the temperature-sensitive mutation that induces the lysogen at 37 °C, an experiment was carried out in which Agarose-eluted fragment 2 was ligated to pSC101 and transfectants were grown overnight at the permissive temperature of 30 °C. In this case, 4 out of 600 transfectants were found to be positive by the hybridisation test. The plasmids prepared from three colonies contained a single copy of fragment 2, whereas the fourth clone contained two copies of this fragment.

It may be interesting to remark that recombinant plasmids containing λ fragments may themselves be useful in that they represent new vectors carrying restriction sites brought in by the λ fragments which may not exist in the parent plasmids.

Figure 1 presents the gel electrophoresis results obtained with the recombinant plasmids described above, before and after *EcoRI* digestion.

These experiments were carried out in low-risk containment conditions, as defined by the Asilomar guidelines. All the clones described in the present paper are available on request.

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Azide-dependent mutants in *E. coli* K12

SODIUM azide is known specifically to inhibit or activate a number of enzymatic activities from bacteria¹ and from higher organisms^{2,3}. Furthermore it has been shown that azide can inhibit the Mg^{2+} -dependent ATPase of *Escherichia*

Table 1 Classification of *E. coli* mutants

	I (ADA)	II (ADB)	III (ADC)	IV (ADD)
Number of mutants	1	1	3	3
Azide replaced by	Nothing	Nothing	Threonine or pyridoxine	Phenylalanine, tyrosine and tryptophan*
Location of the mutation (Taylor's map)	Around 7 min†	Around 20 min†	0 min- <i>thrC</i>	Around 5 min†

The parental strain is CSH 57A (ref. 6). Ultraviolet mutagenesis (10^{-2} – 10^{-3} survival) was used.

*The parental strain is Trp⁻. The conditional requirement for all three aromatic amino acids was tested on an ADD mutant which received the wild-type *trp* operon by transduction.

†Approximate mapping determined by interrupted conjugation.

*coli in vivo*⁴, at concentrations that do not produce any deleterious uncoupling effects on the membrane energy-transducing apparatus of the cell⁵. We felt that those facts could provide the basis for searching out a new type of mutation in *E. coli*—azide dependency.

The selection procedure involved penicillin treatment in a liquid minimal medium devoid of azide, followed by a second enrichment step given by the presumed slower growth of the mutants on a solid minimal medium containing 2×10^{-3} M azide. Eight independent mutants were isolated and separated into four classes according to the specific replacement of azide for growth and the location of the mutation on the *E. coli* chromosome (Table 1).

Two of the mutants (ADA 1 and ADB 1) have an absolute requirement for azide. Although they exhibit an identical phenotype, conjugation experiments show that they have distinct locations, mapping at about 7 and 20 min respectively on the *E. coli* chromosome. The ADA 1 mutation was shown to be recessive to the wild-type allele. If exponential cultures from either mutant strain were transferred in growth medium devoid of azide all growth ceased in about two hours. Measurements of the kinetics of incorporation of tritiated uracil and ¹⁴C-L-tryptophan in TCA

insoluble material *in vivo* suggest a translational defect in both mutants.

The third class of mutants (ADC 1–3) includes Thr⁻ conditional mutants whose specific requirement for threonine can be replaced either by sodium azide or pyridoxine (Table 1). Mutations were cotransducible at high frequency with *pyrA* and *serB*, and thus presumably mapped in the threonine operon. This was confirmed by the use of a λ phage carrying the entire threonine operon⁷ and three derived λ phages each carrying a mutation in one of the three genes (*thrA*, or *thrB* or *thrC*)⁸: the ADC mutations tested were recessive and mapped precisely in the C gene of the operon Threonine synthase—the C gene product—was then measured in crude extracts from one mutant strain grown on minimal medium containing azide (Table 2). Only 5% of the wild-type specific activity was found in this mutant. The mutant enzymatic activity *in vitro* was not stimulated by sodium azide; on the contrary azide gave a 15–20% inhibition, comparable to the inhibition obtained on the wild-type activity. Kinetics of irreversible thermal denaturation of the enzyme in crude extracts showed monophasic curves for wild-type and mutant activities but with different half lives: about 4.5 min for the wild type and 0.5 min for the mutant at 56 °C. This thermolability of the mutant enzyme compared with its wild-type counterpart seems to be a real property of the modified enzyme since a mixture of mutant and wild-type extracts showed biphasic kinetics compatible with the mere addition of the two separate kinetics. Interestingly enough, in crude extracts, azide potentialised the irreversible thermal denaturation of the wild-type enzyme, but had no such effect—nor any stabilising effect—on the mutant enzyme (Table 2). Once purified, however, the wild-type enzyme (prepared by B. Burr) showed a complex biphasic curve of denaturation kinetics with no measurable effect of azide. Threonine synthase from the parental and the mutant strains was partially purified and the affinity for pyridoxal-5'-phosphate measured. Estimation of dissociation constants for the coenzyme from Hill plots at $v = V_m/2$ gives a value of 1.2 μ M for the wild-type and 8.5 μ M for the mutant enzyme. Consistently, in the absence of any added pyridoxal-5'-phosphate, about 15% of the wild-type enzyme was found to be in the holo-enzyme form whereas no such form could be detected in the mutant. Sodium azide 2×10^{-3} M or 2×10^{-2} M had no significant effect on the dissociation constant of the mutant enzyme for pyridoxal-5'-phosphate.

The last class behaves as pleiotropic conditional mutants. Their growth is dependent either on sodium azide, or on tyrosine, phenylalanine and tryptophan (Table 1). However, shikimic acid does not allow growth. One mutant (ADD 1) was mapped by interrupted conjugation: the gene involved is approximately at 5 min on Taylor's map. Reversion of the Tyr⁻ or Phe⁻ phenotype in all cases tested (more than 100) gave a pleiotropic reversion with the additional property of large excretion of both tyrosine and phenylalanine (reversion rate of about 10^{-5}). Enzymatic activities of the aromatic pathways were measured in crude extracts of two revertant strains grown on minimal medium in the absence of phenylalanine and tyrosine (in the presence of

Table 2 Thermosensitivity of threonine synthase in ADC 1 crude extract

			Wild type	Mutant ADC 1
Half life of thermal denaturation (min)	No addition	56 °C	4.5	0.5
		49.5 °C	8.5	5.5
	with NaN ₃	56 °C	3	—*
	2×10^{-3} M	49.5 °C	—*	6
	with NaN ₃	56 °C	2	—*
	2×10^{-3} M with NaCl 2×10^{-3} M	49.5 °C 56 °C	—* 4.5	5.5 —

*Not done.

Bacteria were grown exponentially in minimal medium containing the growth factors of the strains (including isoleucine), glucose 0.4% and sodium azide 2×10^{-3} M. Crude extracts were prepared by sonication of the bacteria previously washed with a potassium phosphate buffer 2×10^{-3} M (pH 7.2). For threonine synthase assay, final concentrations in 0.1 ml of the reaction mixture were: 50 mM N-2-hydroxyethylpiperazine-N'-2-ethane sulphonate adjusted to pH 8 with KOH; 100 mM ammonium sulphate; 0.1 mM pyridoxal-5'-phosphate; 0.24 mg ml⁻¹ bovine serum albumin; and 1.3 mM (0.035 mCi mmol⁻¹) ¹⁴C-L-homoserine phosphate (prepared according to ref. 9). The reaction was carried out at 37 °C for 5–60 min. At the end of the reaction, 5 μ l of KH₂PO₄ 1 M and 0.9 ml of barium bromide (11 mg per ml 90% ethanol) were added, and the precipitated mixture was allowed to stand for 5 min at 4 °C. An aliquot of the supernatant was counted for radioactivity. The radioactivity found in the supernatant at 0 min reaction time was subtracted from the radioactivity measured (this background represents less than 2.5% of the total added radioactivity). At least 90% of the non-precipitating radioactive product was shown to be threonine by chromatography. The specific activity of the wild-type enzyme was 11.7 nmol min⁻¹ mg⁻¹ of proteins.

For thermal denaturation experiments, NaN₃ (or NaCl) was preincubated with the crude extract for 10 min at room temperature.

Table 3 Activities of the biosynthetic pathways of tyrosine and phenylalanine in ADD 1 mutant and two revertant strains

Growth medium	Wild type * †	ADD 1 †	Revertant I from ADD 1 *	Revertant II from ADD 1 *
Prephenate dehydratase	7.5	8.0	12.5	11.0
Prephenate dehydrogenase	23.5	23.5	103.5	288.5
Chorismate mutases	34.0	33.5	100.0	335.5
DAHP synthetases	159.0	195.0	267.5	512.5

Crude extract preparation was as described in legend of Table 2. Buffer was potassium phosphate 2×10^{-2} M (pH 7.2). Prephenate dehydratase and chorismate mutase assays were done according to ref. 14. Prephenate dehydrogenase assay according to ref. 15. DAHP synthetase assay according to ref. 15. Units are $\mu\text{mol per min per mg proteins}$ ($\times 10^3$).

*Bacteria were grown exponentially on 63 minimal medium containing the growth factors of the strains and glucose 0.4%.

†Bacteria were grown exponentially on the above medium containing in addition sodium azide 2×10^{-3} M.

tryptophan) (Table 3) Relative to their wild-type counterparts, prephenate dehydratase activity, an enzyme specific to the phenylalanine pathway, was found to be 2.5 higher in strain I and 1.5 higher in strain II; prephenate dehydrogenase, an enzyme specific to the tyrosine pathway, showed an increase of about 12-fold in strain I and 2.5-fold in strain II, chorismate mutases, specific both for phenylalanine and tyrosine, increased about 10 times in strain I and 2.2 times in strain II. As to DAHP synthetases, the increase in both strains was more than threefold, being due largely (but not exclusively) to the tyrosine-sensitive DAHP synthetase increase. It should be noticed that the above enzymes concern three different operons¹⁰⁻¹². ADD 1 mutant, grown in the presence of NaN₃, similarly showed a significant increase in the above activities compared to the wild type grown in the same conditions (Table 3).

In summary, mutations in at least four different genes can result in azide dependency for growth. None map in the described *azi* gene which is responsible for azide sensitivity^{17,18}. Two genes, at distinct locations, appear to be concerned with the translational mechanism of the cell. No known genes of this type in *E. coli* have the required locations. The third class of mutants map in the *thrC* gene, and the fourth class gives rise to a conditional pleiotropic requirement for tyrosine, phenylalanine and tryptophan which has not been previously described.

Although the precise step or mode of action of sodium azide is still to be found, the search for azide-dependent mutants in *E. coli* seems to be rewarding, having given rise to the discovery of three possible new genes and one new allele of a known gene, and perhaps permitting the detection and study of other new genes and new gene products.

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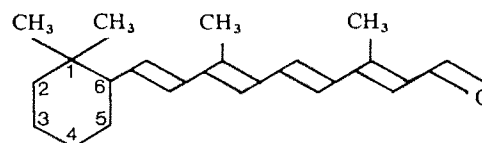
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Is proton transfer the initial photochemical process in vision?

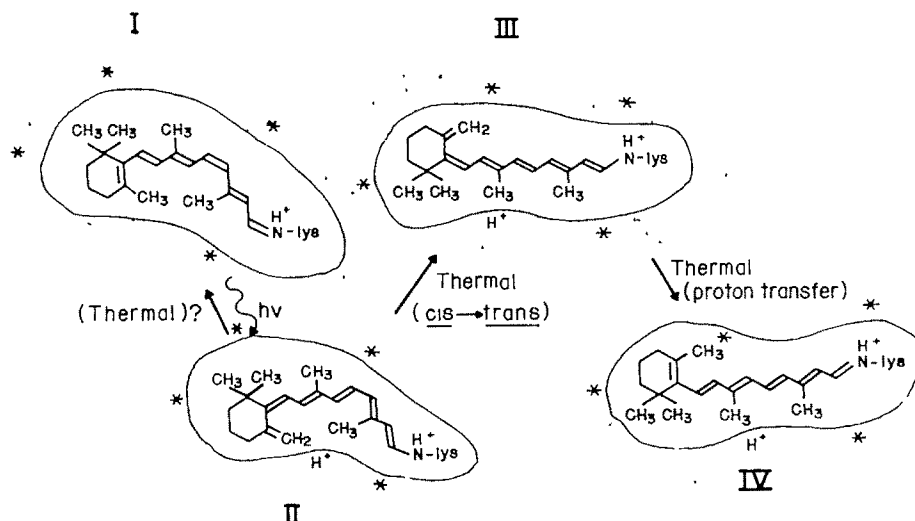
THERE has been renewed effort¹ to test the so-called proton transfer hypothesis (PTH)² or hydrogen shift¹ formulation for the initial photochemical step in vision. This hypothesis proposes an alternative to photochemical *cis* to *trans* isomerisation of rhodopsin, while attempting to account for some of the spectral properties of bathorhodopsin (previously called prelumi-rhodopsin), particularly certain low frequency lines at 920, 877 and 856 cm⁻¹. Spectral lines in this region are not unique to the resonance Raman spectrum of bathorhodopsin³, however, for similar spectral absorption has been found with 11-*cis* retinal crystals⁴. The discovery that the batho-intermediate formed by irradiating chicken iodopsin at -195 °C spontaneously reverted to iodopsin at -140 °C by a dark, thermal process⁵, rather than decaying to lumiodopsin, also seemed to require such an alternative hypothesis² (Fig. 1). Although an attempt to demonstrate proton transfer by means of tritium incorporation into the retinylidene chromophore failed², Fransen *et al.* apparently now confirm the PTH by their report¹ of a small, though significant incorporation of deuterium into retinal. Their newer experiments are similar in design to the earlier ones, differing mainly in their use of deuterium rather than tritium and their method of analysis of labelled retinal. The experiments described here bear directly on the proposed PTH and were performed with an analogue of retinal, 5-desmethyl (dm) retinal:



They show that the initial compound produced by irradiating a visual pigment, the batho-intermediate, cannot result from the transfer of a proton from the chromophore to opsin.

5-dm retinal was prepared during a study of the spectral properties of visual pigment analogues⁶. The presence of a chromophore bearing a hydrogen atom, rather than a methyl (CH₃) group, on the 5 position of the ionone ring

Fig. 1 Hypothetical mechanism for photochemical proton transfer followed by thermal *cis* to *trans* isomerisation in a visual pigment. The native or regenerated visual pigment, is represented by structure (I). In the experiments referred to^{1,2}, * represents either deuterium or tritium atoms which have been introduced into opsin by incubation in either deuterated or tritiated water, respectively. After photochemical transformation to the batho-intermediate (II), incorporation of deuterium or tritium into the retinylidene chromophore can occur during either the thermal steps leading to the intermediate (IV), or in the hypothetical thermal return of the batho-pigment to (I). Presumably intermediate III is a short-lived transient.



should preclude the formation of the proposed batho-intermediate¹. In particular, the structure designated as (II) in Fig. 1 is impossible in a pigment containing the 5-dm chromophore.

5-dm retinal was synthesised as described earlier⁶ and then purified by high performance liquid chromatography (HPLC). Individual geometric isomers were isolated by HPLC following the procedure and with separations similar to those reported for the isomers of retinal⁷.

Identification of the 5-dm retinal isomers was based on their photochemical behaviour as well as their order of elution from silica gel, which seemed to be the same as that of *cis* and *trans* retinals. The spectral maxima of the individual 5-dm isomers are given in Table 1.

Table 1 Spectral maxima of all-*trans* and mono-*cis* isomers of 5-dm retinal

	(nm)	(nm)
all- <i>trans</i>	373	268 (0.13)
13- <i>cis</i>	368	268 (0.29)
11- <i>cis</i>	367	268 (0.67)
9- <i>cis</i>	367	273 (0.34)

The values given in parentheses are relative heights of the secondary peaks. For each isomer, the main absorption band is arbitrarily assigned an absorbance of 1.0 at the wavelength of maximum absorption. The solvent was hexane.

Each of the isomers was mixed with a digitonin solution of opsin which had been prepared from frozen cattle retinae⁸. Only the 11-*cis* and 9-*cis* isomers combined, in each case forming a pigment with λ_{max} at 480 nm but at rates approximately 100 times slower than observed with the corresponding isomers of retinal. Complete spectra and other properties of these artificial pigments, called 5-dm rhodopsin (11-*cis*) and 5-dm isorhodopsin (9-*cis*) will be presented elsewhere.

To determine the presence and properties of any intermediates during the photolysis of the 5-dm pigments, I studied the photochemical behaviour of 5-dm isorhodopsin at low temperatures, utilising the absorption vessel designed and built by Yoshizawa and Horiuchi⁹ for work at liquid nitrogen temperatures and above. 5-dm isorhodopsin which had been formed by reacting 9-*cis* 5-dm retinal with a digitonin solution of opsin was mixed thoroughly with twice its volume of glycerol. Measured in a 0.3-cm light path cell at 20 °C, the absorbance of the resulting solution was 0.5 at 480 nm. Figure 2a, curve 1 is the spectrum of 5-dm isorhodopsin at 191 °C. Some unreacted 9-*cis* 5-dm retinal remains as the oxime. Spectra resulting from violet light irradiation of the pigment in glycerol-aqueous glass are

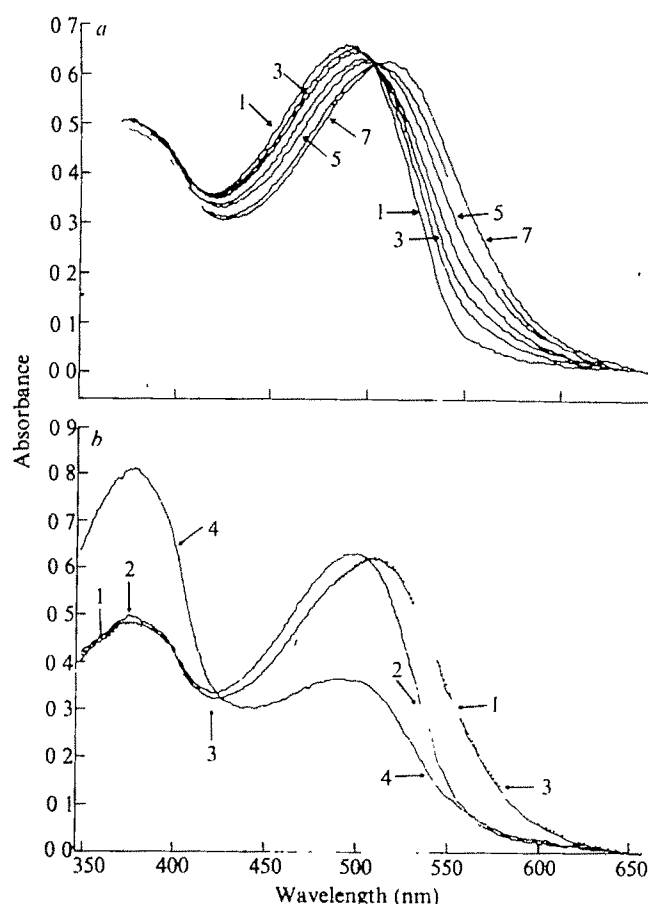


Fig. 2 a, The photochemical transformation of 5-dm isorhodopsin to 5-dm bathorhodopsin. 0.3 ml of a 67% glycerol solution of regenerated 5-dm isorhodopsin in approximately 1% digitonin and 0.01 M NH_4OH , pH 6.5, was placed in a cell with a 3-mm light path. The sample was cooled to -191°C and its spectrum recorded (1). The sample was then illuminated with violet light formed from the radiation of a high intensity xenon lamp which was passed through a 437-nm interference filter. Spectra 2-7 were recorded after total irradiation times of 2 s, 40 s, 80 s, 160 s, 320 s and 1,280 s, respectively. b, The reversible photochemical transformation among 5-dm batho-, 5-dm iso- and 5-dm rhodopsin. Conditions are the same as for a. 1, Same as spectrum (1) in a; 2, after irradiation with red light (600-nm cutoff filter) for 6.5 min; 3, after irradiation of (2) with violet light (437-nm interference filter) for 8 min; 4, after warming solution to $+20^\circ\text{C}$ and then recooling to -191°C .

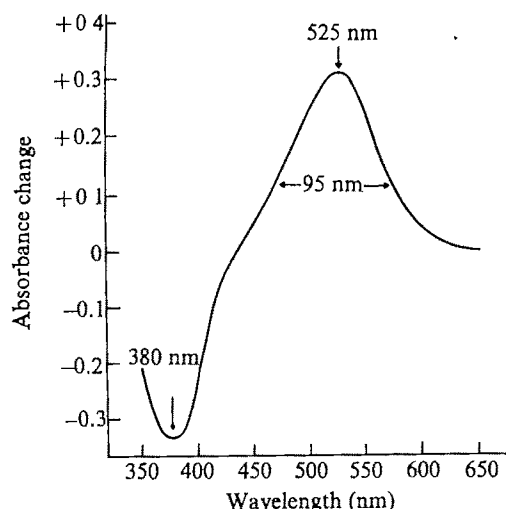


Fig. 3 Difference spectrum of 5-dm bathorhodopsin at -191°C . Conditions are given in Fig 2a and b. The curve drawn results from subtracting spectral curve (4), from curve (3) in Fig. 2b.

shown in Fig. 2a, curves 2–7. The appearance of an isobestic point at 503 nm argues that Fig. 2a illustrates the single-step transformation of 5-dm isorhodopsin into one, or a mixture of, long-wavelength absorbing intermediate(s).

Photoreversibility of this pigment system is shown in Fig. 2b, first by the transformation of the long wavelength absorbing intermediate(s) into shorter-wavelength-absorbing species, presumably 5-dm rhodopsin and 5-dm isorhodopsin. The first process (curves 1 and 2) is followed by the reformation of the long wavelength absorbing intermediate(s). Thus spectrum 3 of Fig. 2b contains thermostable pigments, presumably 5-dm rhodopsin and 5-dm isorhodopsin, as well as a thermally unstable component which decomposes to 5-dm retinal oxime and opsin when the solution is warmed to 20°C , as shown in spectrum 4. The difference spectrum of the thermally unstable compound, drawn in Fig. 3, has a positive peak with λ_{max} at 525 nm and a band width at half maximum of 95 nm. This spectrum characterises an intermediate which, because of its spectral and thermal analogies to cattle bathorhodopsin⁹, I call 5-dm bathorhodopsin.

When the violet irradiated mixture of 5-dm pigments (Fig. 2b, curve 3), containing 5-dm bathorhodopsin, and presumably 5-dm rhodopsin and 5-dm isorhodopsin, is warmed to only about -165°C , a decrease in absorbance at wavelengths greater than 500 nm and an increase in absorbance in the 400–500 nm region occurs. This change is consistent with the transformation of 5-dm bathorhodopsin into a pigment with λ_{max} at 497 nm and band width at half maximum of 80 nm. I call this pigment 5-dm lumirhodopsin. The spectrum of 5-dm lumirhodopsin and its formation from a longer wavelength precursor at around -165°C suggest it is analogous to cattle lumirhodopsin¹⁰.

These results show that a batho-intermediate can be formed from a pigment lacking an allylic methyl group in the ionone ring of the chromophore. Without such a methyl group it is impossible to transfer a hydrogen atom or ion from a group on the ionone ring to opsin and thereby form the structure proposed^{1,2} for bathorhodopsin. Since 9-dm pigments and 13-dm pigments also form batho-pigments at -190°C (my unpublished results), it seems reasonable to conclude that photochemical proton transfer from an allylic methyl-group on the chromophore to a site on opsin is not responsible for the rhodopsin (or isorhodopsin) to bathorhodopsin transition. *Cis* to *trans* isomerisation

still seems to be the process which can best account¹¹ for the experimental observations bearing on the initial photochemical event in the photochemistry of visual pigments, both natural and artificial.

This work was supported by a grant from the National Eye Institute. The low temperature experimentation was performed while I was a visiting professor in the Department of Biophysics of Kyoto University and I thank Professor T. Yoshizawa and his colleagues for hospitality and assistance. I also thank Professor B. Honig, Hebrew University of Jerusalem, for encouragement and R. B. Kropf for helpful emendations in the manuscript.

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Corrigendum

In the article “Perception of melodies” by H. C. Longuet-Higgins (*Nature*, **263**, 646; 1976) there are some errors in the figures. In Fig. 3 the top left-hand number should be -9 ; in Fig. 4 the second x in the left-hand column should be a z ; in Fig. 7 the trilled minim should be prefixed by a $\#$.

Errata

In the article “Male emigration and female transfer in wild mountain gorilla” by A. H. Harcourt *et al.* (*Nature*, **263**, 227; 1976) the second author should be K. J. Stewart and not as printed.

In the article “Axial, magnetron, cyclotron and spin-cyclotron-beat frequencies measured on single electron almost at rest in free space (geonium)” by R. Van Dyck, Jr, P. Ekstrom and H. Dehmelt (*Nature*, **262**, 776; 1976) $(n+\frac{1}{2})\nu_0$ in the first equation should read $(n+\frac{1}{2})(\nu_0-\nu_m)$. In the acknowledgment, for Schwingberg read Schwinberg.

Nature Index and Binders

The **Index** for 1975 is now available, price £2.25. Copies of the 1974 index are still on sale, price £3.00. **Binders** for the journal are also available at £8.00 for four (a year of *Nature* fits into four binders).

Postage is included in the above prices. Orders should be sent, accompanied by remittance to Macmillan Journals Ltd, Brunel Road, Basingstoke, Hampshire, England.

reviews

Secrets of the Earth's core

Thomas H. Jordan

The Earth's Core. (International Geophysics Series.) By J. A. Jacobs. Pp. viii+253 (Academic: New York and London, 1976.) £8.50; \$22.

THE Earth is stubborn in yielding her secrets, and locked most tightly in her depths are the mysteries of her core. The core is separated from us by nearly 3,000 km of rock, so it is no wonder that our papers about its composition, state and dynamics are often prefaced by words like 'assumption', 'hypothesis' and 'paradox'. Pressures within the core are as yet inaccessible by static laboratory apparatus, but fall short of the domain in which the elegant theories of degenerate electron gases may be applied. Nearly everyone agrees that iron and nickel are the dominant atomic species in the core, but further statements about its composition depend heavily on models for the Earth's early history, a subject perhaps still better suited for religious thinkers than natural philosophers. The important questions of core dynamics seem to provide the most secure remaining refuge for the back-of-the-envelope, order-of-magnitude geonomist. For example, the average heat flux across the core-mantle interface is one of the great unknown quantities of terrestrial physics, a number whose demonstrated bounds are virtually useless.

Therefore, few geoscientists would be surprised to learn that, until now, the Earth's core has not been the title subject of a scholarly treatise. This deficiency has been remedied by Professor Jacobs, who has produced a very readable monograph of 253 pages. His narrative sketches the lines of evidence which, when suitably tangled, form the web of our understanding about the core. His treatment of this subject has been divided into five chapters the titles of which outline the content of the book: general physical properties of the Earth, origin of the core; thermal regime of the Earth's core, Earth's magnetic field; constitution of the core. As a bonus he concludes with a sixth chapter on the cores of other planets, paying due homage to the current emphasis on comparative planetology. This last chapter also serves to cheer us up; at least we Earth scientists know

this planet has a core, a conclusion that is uncertain for even the best studied of our neighbours.

The book is comprehensive: space is given to nearly every subject of current interest, although few are treated in any depth. Particularly valuable are the author's discussions of recent attempts to model the physical parameters of the core using seismic methods, his summary of the controversy that has surrounded the so-called Kennedy-Higgins paradox, and his synthesis of the evidence regarding core formation. The chapter on the magnetic field is adequate, although a more thorough treatment of the dynamo problem could have been attempted.

Throughout the book the mathematical level is very elementary, long derivations are absent, and equations are generally used only in a narrative

fashion. In spite of this lack of fancy mathematics, or perhaps because of it, the subject matter is modern and the information content is high. It is a good reference for specialists engaged in core research who wish to know what other specialists are up to. The bibliographies which follow each chapter are excellent, with nearly seven hundred references (including multiple citations), over half of which have been published since 1970.

This book should be an excellent text for graduate courses dealing with the internal constitution of the Earth. It will be interesting to see if, in five or ten years, this is still true. □

Thomas Jordan is Assistant Professor at the Scripps Institution of Oceanography, La Jolla, California.

Introduction to physiological psychology

Brain and Behaviour: A Textbook of Physiological Psychology. By Hugh Brown. Pp. x+413. (Oxford University: New York and London, March 1976.) £9.25.

THIS introduction to physiological psychology has grown out of a series of lectures for undergraduates. The author initially grapples with the problem of providing a balanced grounding in the principles of nervous function and I think he succeeds in his aim of holding the attention of the reader while proceeding at a somewhat breathless gallop through the first half of the book. The text is chatty and is liberally punctuated with anecdotes, photographs and excellent illustrations. Jargon is kept to a minimum and there is a glossary of terms at the end, ranging from ablation and acalculia to Witzelsucht and the Young-Helmholtz theory. Each chapter ends with a few well chosen suggestions for further study.

The reader is then propelled rapidly through, and sometimes over, the second half of the book, which covers a selection of behavioural correlates of physiological functions. There are sections of about 20 pages each on motivation, emotion, consciousness, frontal lobe localisation, learning,

personality and psychopathology; and then on to glimpses of social and aggressive behaviour, wanted and unwanted actions of drugs; and the book comes to a close with a brief discussion of the mind-body problem. Comments on the composition of this section must, to some extent, reflect personal biases; I missed any discussion of recent attempts to link neurotransmitter systems with reinforcement mechanisms, and the neuropharmacology of mental disorders receives scant attention. There are occasional jarring lapses into simplistic clichés—for example, "if mental illness could be reduced to a chemical problem . . ."; and, incidentally, imipramine is not a monoamine oxidase inhibitor.

The book is largely successful in its aim; it is interesting but not indigestible. As an hors d'oeuvres the basic ingredients are well selected, nice to look at, and they whet the appetite. One big snag is the price—an hors d'oeuvres at £9.25 probably means that most students would have to do without the main course.

R. Kumar

Dr Kumar is a Senior Lecturer in the Institute of Psychiatry, London, UK.

Behaviour genetics

The Genetics of Behavior. By Lee Ehrman and Peter A. Parsons. Pp. viii+390 (Sinauer Associates: Sunderland, Massachusetts; Freeman: Reading, June 1976.) £11.50.

BEHAVIOUR genetics is not easily organised into textbook form, as it's literature covers a very diverse range of topics in a patchy fashion. Ehrman and Parsons have chosen to group their material according to experimental approach and experimental animal. There are chapters on 'single genes and behaviour', 'many genes and behaviour', 'Drosophila', 'Rodents', 'other creatures', 'man—continuous traits', 'man—certain discontinuous traits', and so on. Their decision is reasonable but it presents them with problems, for such groupings are not the best for bringing out the main questions to which behaviour genetics should be applied. This book was written just too early to have taken account of the sociobiology upsurge. Here, for example, we have an infinity of attractive theories confidently proposing a genetic basis for a huge range of complex social behaviour. Yet we know next to nothing about the way the simplest behavioural potential is encoded in genetic terms. This main question quickly breaks down into a cluster of questions concerning genes and behavioural development, and gene environment interactions. Of course, these and other major questions do emerge from Ehrman and Parsons's book, but I think they should have been more strongly accented, to give some structure to their review of the very disparate literature, only a few of whose studies really lead somewhere.

This will, however, be a most useful textbook; it is certainly the best since Fuller and Thompson produced the first complete survey of behaviour genetics in 1960. Ehrman and Parsons write very well, their style is generally light and attractive. They begin with a chapter of essential genetics and I would judge that even students with no biological background could then go on with little trouble. The examples they cite are described thoroughly, with good illustrations and tables. Although I might quibble with the balance in places, they have certainly drawn together a scattered literature with great skill. Both authors are primarily geneticists and at times, I find the behavioural component lacking in depth. For example, their accounts of Dilger's work on hybrid parrots and Sharpe and Johnsgard's duck hybrids miss some of the points.

The chapters on human behaviour genetics are very good. The real test

here is the treatment of the genetics of intelligence issue and this is the best modern account I know, steering a very clear and reasonable course, missing none of the genetical or social issues.

Ehrman and Parsons end with a chapter on the evolutionary implications of behavioural changes, and then finally give their appraisal of future directions for behaviour genetics. I don't really share their conviction that it will emerge as a distinct discipline, but genetics will remain a vital technique in tackling some of the most fundamental behavioural problems. This textbook can be warmly recommended to teachers and students alike.

Aubrey Manning

Aubrey Manning is Professor of Natural History at the University of Edinburgh, UK

Ice on the land

Glaciers and Landscape A Geomorphological Approach. By David E. Sugden and Brian S. John. Pp. viii+376 (Edward Arnold. London, April 1976.) Boards £12; paper £5.95.

GLACIOLOGY and geomorphology are typical of many scientific subjects the early disciples of which, with a minimum of equipment, made bold observations from patient work almost always pursued in isolation. Since 1950 the pattern has changed to team work, sophisticated equipment, the sharing of investigation areas and special publications. Devotees from all industrialised nations meet regularly to learn of progress and methods, and to swap yarns of experience, always told with humour and understatement by those who, particularly in these two disciplines, raise their eyes to the hills from 'whence cometh their health'. Laboratory work has provided specific laws but the wide range of variables in natural processes in the field always raises new problems in the application of these laws to the observed landscape.

Glaciology is advanced primarily by physicists whereas geomorphology is lead by physical geographers. The authors are reputable glacial geomorphologists who appeal for investigations to link these two aspects more closely. The book systematically sets out a brief for the geomorphologist to recognise the scope of the ice problem and summarises what the glaciologist has done to assist.

There are five parts, each of three chapters. The first part, 'Glaciers and Glacier Dynamics', gives the basic properties of ice, the environmental factors influencing it, the morphology of

glaciers both *en masse* and in surface structure. Fundamental quantitative relations are limited to this first part.

'Glacier Distribution in Space and Time' provides a succinct summary of the ice on the Earth's surface and its fluctuations. The authors rightly criticise the overdramatisation by the media of the relation between glaciers and climate and conclude that reliable assessment of this relationship can only be made for high frequency events of low magnitude since precise data is only available for minor oscillations over the past few decades. The random element in oscillatory behaviour becomes more marked as the time scale increases.

The next two parts, 'Glacier Erosion' and 'Deposition', each give a chapter on process followed by ones on landforms and landscapes. Rock abrasion, meltwater transport and deposition of rock detritus, the characteristics of tills and the complex end-products of both processes are economically described. Meltwater phenomena extend well beyond the margins of present ice but the authors sensibly limit their account to the present glacierised phase.

The final part, 'Meltwater, a glacial subsystem', discusses a component in all glaciers that in recent years has received much attention from glaciologists and engineers. It is a very creditable account of an aspect that most authors have combined with erosion and deposition. As in the first part of the book, there is already new material to be incorporated, a common difficulty for authors in a subject developing so rapidly.

Each chapter ends with suggested 'Further Reading' and there is an index at the end of the book, preceded by 25 pages of references, since the authors have patiently sifted an extensive range of literary and photographic material, very well drafted and printed in the book. Almost every page has a diagram, a photograph, a table, a schematic model; and although the models are entirely qualitative and the authors admit that some will rapidly be superseded, most are new and will be welcomed by students being increasingly taught classifying systems.

Few books are better presented for the price and none more captivating to the reader interested in the fascinating phenomenon of ice on the land.

H. Lister is Reader in Physical Geography at the University of Newcastle upon Tyne, a member of the International Glaciological Society and of the British Geomorphological Research Group, and escapes to mountains and glaciers whenever possible. **H. Lister**

Dr Lister is a Lecturer in the Department of Geography at the University of Newcastle-upon-Tyne, UK.

obituary

Eric Higgs, who died in Cambridge on September 23 after a long illness, will be remembered especially for his contribution to economic prehistory over the past decade. He became an archaeologist at a time of life when most think only of retirement, after a first career as an economist and a second as a hill farmer. The impact that he made in his third career, as a research and teaching archaeologist at Cambridge University, is all the more remarkable for the fact that his health was poor—intermittently at first, but increasingly so with time.

He found prehistoric archaeology dominated by the belief that the key to understanding early man rested in the study of his artefacts and of the cultural groupings based on artefactual styles: the relationships between the prehistoric cultures were the cornerstone of the subject, largely divorced from the natural environment, and the ordinary economic life of prehistoric communities had been relegated by most prehistorians to a comparatively minor role in the history of early man. The study of prehistoric subsistence consisted of what he termed a 'horrid porridge' of specialist reports which, although embodying a considerable degree of technical competence in the analysis of biological data, usually did little to build an integrated picture of man's relationship with his environment. To Higgs, the emphasis on the importance of cultural data was fundamentally misleading, in that it obscured basic economic motives, such as the need for food, which were and are the vital core of human behaviour. By assigning subsistence to a central place in prehistoric archaeology, he did much to establish economic prehistory as an independent discipline based on its own concepts and supported by its own laboratory and field techniques.

These interests crystallised during his fieldwork in Greece (Epirus) in the

middle and late sixties. In 1967 he was appointed director of a British Academy Major Research Project to study the origins and early history of agriculture. From then until his death he presided over a hectic decade of original and fundamental research and fieldwork, in areas as diverse as Alaska and Israel, on periods as widely separated as the Middle Palaeolithic and the Mediaeval.

This research was important in two ways: a theoretical contribution to the study of prehistoric subsistence in general and early agriculture in particular, and methodological developments in fieldwork. His project undertook a long overdue reassessment of the basic concept of animal and plant domestication. He argued that it was unrealistic to expect that human subsistence in prehistory would divide neatly into hunting and farming; instead, there would have been a series of relationships between prehistoric men and their plant and animal resources. Prehistorians have yet to appreciate fully the implications of this reassessment, for they have yet to accept that when and where agriculture originated cannot be confined to a convenient postglacial Garden of Eden, or that its origins had better be re-defined on more sensitive and complex criteria.

Of the new techniques he introduced, some, such as froth flotation for retrieving botanical remains from habitation sites, or site catchment analysis for quantifying economic potential of the areas exploited from prehistoric settlements, were culled from other disciplines, and modified; others, such as sieving techniques for recovering faunal samples, were already in occasional use but have since become an integral feature of modern palaeoeconomic investigations. This fieldwork, embodied in two major publications—*Papers in Economic Pre-history* and

Palaeoeconomy—was conducted and published within five years and was usually undertaken on field budgets which would have confined many another expedition to the armchair. Higgs even made his students pay 5 shillings a day for the privilege of working 8-h on-off shifts (under floodlights if necessary) at the site. (Hammond Innes did his fieldwork for the novel *Lefkas Man* by watching Higgs in action in Greece.)

Inevitably, his research aroused much debate and criticism. Many of his colleagues felt uneasy about his conviction that prehistoric archaeology had hitherto been concerned with only trivial aspects of past human behaviour and few shared his sublime indifference to the vagaries of ceramic and lithic styles. Yet the simplicity of Higgs's viewpoint was its strength, for it provided a direct challenge to an ageing conceptual framework which had denied the biological basis of prehistoric human behaviour. As Higgs said, "did Man Make Himself and control his own destiny as Childe had taught, or did he, like Topsy, just grow?"

Those who knew him personally, could not fail to recognise him as a man of remarkable character. He was an utter individualist, with scant regard for convention in either his professional or his personal life. It was in keeping with the man that he merged both, and shared them with his students to a remarkable degree; in return he expected and generally received a degree of commitment that often surprised even those who gave it. Eric Higgs was a remarkable man, in particular a gifted and riveting teacher. Prehistoric studies in general, as well as the personal and professional lives of those who knew him, are much the poorer for his death.

Graeme Barker
Robin Dennell

Benjamin Franklin Howell, Professor Emeritus of Geology and Palaeontology, at Princeton University, died on May 28 at the age of 85.

Howell was born on September 30, 1890, in Troy Hills, New Jersey. At the age of 18 he entered Princeton University where he received his Bachelor's degree in 1913, a Master's degree in 1915, and a Doctor of Philosophy degree in 1920, all in the Department

of Geology. He remained at Princeton where he was appointed as Assistant Professor in 1920 and became Professor of Geology and Palaeontology in 1947. He also served as Curator of Palaeontology and Stratigraphy at Princeton from 1924 until his retirement in 1959.

In addition to his work at Princeton, Howell was engaged in adult education as Professor of Geology at the Wagner

Free Institute of Science in Philadelphia, Pennsylvania, from 1928 to 1947. He also served as part-time Curator of Palaeontology at the Academy of Natural Sciences in Philadelphia from 1937 to 1947 and as a Visiting Lecturer in Palaeontology at the University of Pennsylvania.

He was a recognised authority on the palaeontology of the Cambrian Period, the earliest geological period of abun-

dant marine invertebrates. He was one of the founders of the International Palaeontological Union, and served as its Secretary in 1936-1937 and as its Vice-President in 1938. He was also an Honorary Member of the Geological Society of London as well as of the Palaeontological Society of India. At various times he had served as a consultant for the United States Geological Survey, the United States National Museum, the Geological Survey of Canada, the Canadian National

Museum, and the geological surveys of Vermont and Montana.

In America Howell was an active member and officer in both palaeontological and geological societies: as a Fellow of the Palaeontological Society he served as its Secretary from 1931 until 1939 and as President in 1944, and was a member of several of its committees concerned with Cambrian fossils and stratigraphy. As a Fellow of the Geological Society of America he was elected Vice-President in 1945.

During the early 1950s he was Chairman of the Cambrian section of the National Research Council's Committee on Stratigraphy.

During his long career Howell was one of the more prolific writers in the geological profession. His bibliography includes more than 200 titles of geological and palaeontological contributions, dealing mainly with Cambrian marine invertebrates, especially trilobites.

Erling Dorf

announcements

Appointments

Dr Barrie Vernon-Roberts as Professor of Pathology at the University of Adelaide.

Mr A. R. Haly as chief of CSIRO Division of Textile Physics.

Dr D. G. Murchison to a Chair of Organic Petrology at the University of Newcastle upon Tyne.

Professor J. Heslop-Harrison as a Royal Society Research Professor, to be taken up at the University of Wales, Aberystwyth.

Professor G. W. Kenner as a Royal Society Professor at the University of Liverpool.

Professor J. L. Gowans of the University of Oxford will take over as the new secretary of the MRC in April.

Meetings

November 17, **Cumulonimbus Convection**, London (T. M. R. Whitwell, James Glaisher House, Grenville Place, Bracknell, Berkshire RG12 1BX, UK).

January 3-5, **Mathematical Aspects of Homogeneous and Heterogeneous Combustion**, Cranfield Institute of Technology, Bedford (The Secretary, The Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend-on-Sea, Essex, UK).

January 6-7, **Respiratory Gas Transport**, London (The Secretary, The Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend-on-Sea, Essex, UK).

January 17-22, **Gondwana**, Calcutta (Organising Committee, IV International Gondwana Symposium, The Geological Survey of India, 27, Jawaharlal Nehru Road, Calcutta-700016, India).

January 31-February 2, **Cadmium**, San Francisco (The Cadmium Association, 34 Berkeley Square, London W1X 6AJ, UK).

February 7-8 (Chicago) and February 14-15 (Brussels), **Laboratory Testing**

Person to Person

Applications for the Victor Heiser Awards for Leprosy Research are invited to apply to Ms Caroline R. Stanwood, Director, Heiser Fellowship Program for Research in Leprosy, 1230 York Avenue, New York, New York 10021. The awards are for. Postdoctoral Research Fellowships, \$10,000-14,000, applications from individuals or heads of departments, Visiting Research Awards (for specific research at distant institutions), Research Grants (possibly available for specific programmes).

Wanted: 3- or 4-bedroom furnished house in central London, preferably Ealing, from August 1, 1977 to July 30, 1978, in exchange for house in Halifax. Replies to Carl Abbott, M.D., Department of Medicine, Camp Hill Hospital, Halifax, Nova Scotia, Canada B3H 3G2.

M.I.T. professor offers a 12-room fully furnished Victorian home in Brookline, Mass., in exchange for a 7-room (minimum) home within reasonable commuting distance of central London for one year beginning July 1, 1977. Brookline is very near Harvard, M.I.T., and major hospitals; has excellent schools and public transportation. Please reply to Dr Harvey Lodish, M.I.T. 16-435, Cambridge, Mass., 02139.

for Cancer (Robert S. First, Inc., 405 Lexington Avenue, New York, New York 10017).

March 23-25, **Nuclear Physics**, Guildford (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK).

April 4-7, **Atomic and Molecular Physics**, Reading (K. Codling, Conference Secretary, J. J. Thomson Physical Laboratory, Whiteknights, Reading RG6 2AF, UK).

April 4-7, **Low Temperature Biological Microscopy**, Cambridge (Dr P. Echlin, The Botany School, University of Cambridge CB2 3EA, UK).

May 8-12, **Psychoneuroendocrinology**, Atlanta (Dr Richard P. Michael, Department of Psychiatry, Emory University School of Medicine, Atlanta, Georgia 30322).

July 13-15, **Processing, Structure, Properties and Performance of Polymers**, Nottingham (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK).

July 24-30, **Optimal Development and Management of Groundwater**, Birmingham (Dr J. W. Lloyd, Organising Secretary, IAH Symposium, Department of Geological Sciences, University of Birmingham, PO Box 363, Birmingham B15 2TT, UK).

August 22-24, **Scientific Instruments: Their Social and Economic Settings**, Greenwich (Organising Secretary, Department of Navigation and Astronomy, National Maritime Museum, Greenwich, London SE10 9NF, UK).

September 4-8, **Low Energy Ion Beams**, Salford (The Meetings Officer, The Institute of Physics, 47, Belgrave Square, London SW1X 8QX, UK).

September 19-24, **Behavioural Development**, Pavia, Italy (Secretary to the 4th Biennial Congress of the International Society for the Study of Behavioural Development, The Institute of Psychology of the Faculty of Letters and Philosophy of the University, Corso Strada Nuova, 65, 27100-Pavia, Italy).

nature*November 11, 1976*

The cost of Swiss francs

It is fashionable to see the late 1960s as a period when science on a worldwide basis went through a major transition, emerging with a much-changed relationship with society in general. That transition, even if major, was at least fairly gradual and allowed for steady adjustment in values amongst scientists. In later years we may well look at 1976 and decide that here was a second equally drastic transition, but one which occurred in the course of weeks rather than years and which affected only Britain. The continuing weakness of the pound—a result not of irresponsible financial journalism, nor even of sterling's role as a reserve currency, but of long-term industrial inadequacy to compete successfully in overseas or even domestic markets—is precipitating a serious crisis of confidence among many scientists about the will, let alone the ability, of Britain to continue as a pre-eminent scientific nation. Much of this alarm is exaggerated, there is no doubt, and there is a danger that we all think ourselves into a worse crisis than really exists.

The details barely need repeating now. Every cent that the pound drops on the foreign exchange markets adds nearly £200,000 to the money that has to be found by the Science Research Council (SRC) to pay international subscriptions, notably to CERN, the European Space Agency, and the Institut von Laue-Langevin. The CERN subscription, at over £20 million, is by far the greatest. The recent downward trend of the pound has thus meant severe economies for domestic programmes in no way related to high-energy physics, even those of other research councils. It has also meant that Britain has had to put out some very careful feelers to CERN and its member states for sympathetic understanding.

What are the options at Geneva? It would conceivably be possible to pull out of specific projects there by way of economy. There has been recent speculation about dropping out of the Intersecting Storage Rings programme, which provides collisions at the highest energy in the world. But there seems no evidence at all that Britain has either taken or intends to take any such step, which would be the most ham-fisted way of disrupting CERN's operations. The danger is that such speculation,

especially in an international framework, can easily turn into a self-fulfilling prophecy. It is much more likely that SRC, if it fails to convince the Treasury that support for an international treaty should be detached from a national policy on cash limits, will go to CERN to see if there is any way that the UK can reduce its overall contribution while maintaining its membership of all programmes. It goes without saying that to have to do this would hardly enhance our reputation as a partner in European ventures.

It is difficult as yet to predict how our partners in CERN would react to such an approach. No other country seems to be experiencing similar problems; Italy might seem the most vulnerable but pays directly from its Foreign Ministry, which has been in the habit of paying without question. One suspects, however, that there will be genuine support, provided that this is not to be an annual affair. The only difficulty is that the Germans, who might logically be bearers of a greater burden, already contribute 25% of CERN's budget, which is the limit that any country may carry. Thus any load shed by Britain could possibly prove an embarrassment for a smaller country to pick up.

But where in all this is the turning point for British science? Will this not seem a relatively minor ripple in hindsight? There are grounds for thinking otherwise, that the affair could polarise the scientific community to its great detriment. There has, of course, for some time been a substantial "anti-big science" feeling only just below the surface in many sectors of the community; the events of the past few weeks with their resultant stringencies across the board can hardly have helped dissipate this feeling. On the other hand the high-energy physicists now make no secret of dissatisfaction with the way SRC plans to shift emphasis in its domestic funding. In particular many are expressing a new-found concern at the council's growing emphasis on engineering research.

Whatever the merits of either of these points of view, they are symptomatic of dangerous splits and uncreative tensions which could very easily harm Britain's research potential and lead her down a depressing road to second-rate status. □

The loneliness of the original investigator

Ernest Borek of the University of Colorado Medical Center comments on the fate of original research under present funding arrangements

THE curse on science is that its masters demand results they can readily comprehend. Kings and princes once gave what we would call today research grants to their alchemists, expecting gold for their largesse. The alchemist Hennig Brandt serendipitously discovered that if he performed some hocus-pocus on dried urine, he obtained a magic stuff. It glowed in the dark! His patron, a German prince, was not impressed, so Brandt's grants continued to be miserly.

Of course, Brandt discovered phosphorous, and our knowledge of the universal need for this element in the form of phosphates by plants and the consequent incorporation of it into fertilizers has improved the yield of crops, producing wealth over the scores of years, which in value probably exceeds all the gold of the world.

Our situation today is not much different. We are the remittance men of Presidents and Royal Commissions. Science is in the political arena; we are at the mercy of the ringmasters. Charles G. Wilson, a Secretary of Defense whose department controlled enormous funds for research, pithily (if ungrammatically) aphorised the opinion of many upper level administrators on research: "Basic research is when you don't know what you're doing".

Five years ago the President of the United States decided, it being a pre-election year, to sign the "Conquest of Cancer Act of 1971", and did so with a flourish. He invited a horde of television men, some legislators, and a sprinkling of scientists to witness the great moment. I am ashamed to admit that curiosity impelled me to accept the invitation. In those pre-Watergate days, I was almost touched when the president told us about his dear aunt who died of cancer. In less than a year he impounded a hundred million dollars from cancer research provided by the bill he signed with so much emotion.

The British scientist is also not free from the paralysing shackles of authority. A few months ago, a young Englishman spent a year in my laboratory learning the skills to study the modifications of tRNA. He went home filled with plans and enthusiasm. He actually made an observation which could have been developed into something very interesting. But soon I received a sad letter from him. The

director of his research institute ordered him to drop studying modification of tRNA because the molecular biologist on some high MRC committee put a very low priority on such research. Chalmers were in vogue.

Now this is hearsay and may be just sour grapes, but I could count on the fingers of one hand the British colleagues contributing visibly to this field. For the benefit of those who are not molecular biologists, I should point out that the primary structure of tRNA is made by one enzyme, but more than fifty modifications of it are achieved by perhaps 150 different enzymes. We know the functions of some of these modifications, but for most of them, the gold is not yet visible. tRNA is the most versatile of the biomacromolecules. In addition to its pivotal participation in protein synthesis, it can regulate transcription of DNA, translation of messenger RNA and can even control enzyme activity. What new roles will be revealed by patient probings cannot be predicted.

The American scientist who strives for originality is bedevilled at two levels of the governance of science. The General Accounting Office (GAO), which is the administration's watchdog over the budget, has recently urged Congress to require annual reviews of all research grants awarded by the National Institutes of Health (NIH). One can just see the staff at GAO with their tidy accountant's minds fretting over the possibility that some scientist may goof off for three years. After all, the corporations from where they came give quarterly reports of their earnings to the stockholders.

Unfortunately, at NIH the administrators urge the appointment of young investigators to peer review groups. James Watson is the most vocal champion of youth on advisory bodies because, he says, creativity is exclusively in the provenance of youth. This may be true in mathematics, but has no factual basis in the biological sciences. A young scientist becomes visible in biological research at an early age not by original major contributions but by frenetic publishing in a field opened by the originality of others. Polish a little and publish a lot, that is the leitmotif of their research careers. "Peers" like that cannot possibly appreciate a new idea or a new approach. At the end of a two- or a three-year grant period,

they measure accomplishment by counting pages rather than by the increment in our knowledge. The grant application of one of our most distinguished cell biologists went unfunded recently; the reason, he was told, was that his proposed experiments were too bold.

The members of advisory panels at the National Science Foundation (NSF) tend to be more mature and accomplished. But Congress insists that grants be awarded for only two years. How can one pose a challenging question and dare explore it when within a year it is time to write the grant renewal? At a recent conference on nucleic acid modification I noted that pre-eminence in the technology of tRNA modification has passed from America to Germany and Japan, and the reason must be that no one in his right mind would dare tackle such a problem on a two-year NSF grant (*Nature*, September 9, 1976).

Some 25 years ago I became engrossed in Lwoff's discovery of lyso-genic induction. In four years, we filled notebooks with observations I did not think were worth publishing. Had I depended on our current system of research support, I would have been washed out. Yet it is a matter of record that from those four years of observation came the discovery of "relaxed control" of RNA synthesis, indirect induction of latent virus, post-synthetic modification of nucleic acids, and the first qualitative difference in a biochemical component of a tumor versus a normal cell.

To beat the system some investigators resort to artful dodging. You describe in your grant application your second level ideas, or experiments you have already done and thus gain time and resources to bootleg explorations of your pet ideas. Five years ago I foolishly described, among other things, a programme of study in tissue slices or *in vitro* of the functional effects of hormone-induced modifications of tRNA. The critique relayed to me was that I had no experience in that field and the funding was proportionately reduced. Needless to say, I went on to do what I wanted (see *J. Biol. Chem.*, **248**, 762; 1973, and *Nature*, **262**, 62; 1976). During these bootlegging operations, we observed serendipitously a novel attribute of the carcinogen ethionine (see *Nature*, **259**, 588; 1976).

Many years ago while an undergraduate, I supported myself and my family as a waiter in a restaurant where one of my duties was selling bootleg whiskey. Now I bootleg my research. *Plus ça change . . .*

Whither US science now?

Colin Norman surveys the post-election scene from Washington

WHEN it became clear shortly before dawn last Wednesday that Jimmy Carter had the electoral votes needed to put him into the White House, many Americans switched off their television sets and went to bed knowing who their next President would be, but not what he will do. Substantive policy matters were almost submerged in a flood of trivia during the long, tedious election campaign as the candidates' many rhetorical blunders, racist remarks of Cabinet members and non-political matters such as abortion claimed the spotlight. Although the campaign lasted many months and cost millions of dollars, it seems to have left most voters puzzled about where the candidates stand on the major issues.

Nevertheless, it is evident that Carter has offered the American people a very different political philosophy from the one which prevailed during the Nixon and Ford Administrations. As the Carter Administration takes shape over the next few months, it is likely to chart new courses in areas such as health policy, taxation, nuclear power, environmental protection and government reorganisation.

Little part for science

Needless to say, scientific issues played little part in the election campaign. Science policy is scarcely a partisan matter, and it hasn't figured prominently in election rhetoric since the Kennedy campaign, which took place in the post-Sputnik panic of the late 1950s. But when Carter takes office next January, he will be confronted with a number of important decisions which will have direct impact on science and technology.

First, however, it should be noted that Carter will enter the White House with at least lukewarm support from the scientific community. Though few scientific groups campaigned actively in support of Carter—in previous elections, groups of scientists campaigned for Kennedy, Johnson and McGovern—opinion polls taken among American academics found that Carter was favoured by a margin of two to one. A poll taken for the *Chronicle of Higher Education*, for example, found that 54% of the scientists surveyed favoured Carter, compared with 22% for Ford.

Carter's first chance to strengthen (or, indeed, to disrupt) his links with the scientific community will probably be in his appointment of people to fill

top science posts in his new Administration. Nearly 3,000 government posts are filled by Presidential appointment, and thus a change of President is usually accompanied by a wholesale change of top-level bureaucrats.

It is worth noting that Richard Nixon amply demonstrated the potential for souring relations with the scientific community in such matters. Early in his Presidency, Nixon vetoed the selection of Franklin Long as Director of the National Science Foundation because of Long's opposition to the anti-ballistic missile programme. Though the veto was made before Long's selection was announced, word filtered out and a shrill outcry was raised against political screening for that normally apolitical post. And Nixon incurred the wrath of many scientists four years later when he sacked the head of the National Institutes of Health, allegedly for disagreeing with White House policy on biomedical research.

Thus, Carter will probably tread carefully. His chief selection will be somebody to fill the post of science adviser and director of the newly-created White House Office of Science and Technology Policy (OSTP). Since OSTP was established by legislation, Carter is bound to retain it—indeed, he has already said that his science adviser will "provide a permanent and high-level relationship between the White House and the scientific community". The present incumbent, Guyford Stever, said when he was appointed last August that he would not stay long after the election, no matter who won. A widely-tipped candidate for the post is Lewis Branscomb, former director of the National Bureau of Standards and now head of research at IBM. Branscomb coordinated a science policy task force which advised Carter during the campaign.

The other chief science posts that Carter must fill are Director of the National Science Foundation and Director of the National Cancer Institute. The NSF post was vacated by Stever when he joined the White House staff and the NCI job was vacated in September by Frank Rauscher, who took a more lucrative job at the American Cancer Society. Carter is also expected to replace Russell Train as head of the Environmental Protection Agency. Though Train is generally considered to have



Carter: important decisions ahead



Ford: departure leaves vacancies

done a good job, the post is highly political and Carter made considerable mileage from attacking the Ford Administration's environmental record. On the other hand, Carter is expected to retain Donald Fredrickson as director of the National Institutes of Health. A skilled administrator, Fredrickson has earned a solid reputation and Carter is unlikely to risk provoking an outcry by replacing him for political reasons.

Carter will also inherit a top-level committee, chaired by Simon Ramo of TRW Inc, which was established by Congress to conduct a two-year investigation of federal science policy. The committee is scheduled to make its report and recommendations late in 1978, at which time Carter will have the option of retaining it as a senior White House advisory committee, or scrapping it.

Key policy decisions

As for the Carter Administration's likely policies for research and development, little is known with certainty, but many key policy decisions will have to be made in the

first few months after President Carter takes office. The following are some of the principal issues which Carter must decide.

● **Energy policy.** One area in which Carter has frequently attacked the Ford Administration's record is energy policy in general, and nuclear policy in particular. He has offered some specific plans for reorganising the federal energy bureaucracy, and for curbing nuclear proliferation.

Throughout the campaign, Carter made much of the fact that he was trained as a nuclear engineer, and therefore he claims to understand the problems associated with nuclear power. His attitude toward nuclear energy is decidedly cool, promising to minimise the United States' dependence on that source of power, but specifically stating that he does not support a nuclear moratorium. In particular, he has said that he will channel more energy research and development funds into alternative sources such as solar energy and geothermal heat. He has also promised to launch an aggressive energy conservation programme.

Carter has also offered a proposal to consolidate most of the departments and agencies now responsible for energy programmes into a single department. Included would be the Energy Research and Development Administration (ERDA)—which Carter has attacked as being overwhelmingly pro-nuclear—the Federal Energy Agency and the Energy Resources Council. The proposal is likely to meet stiff opposition in Congress, however, since many Congressional committees which now have jurisdiction over individual agencies would be wary of losing some of their authority. A prolonged battle should be anticipated.

On nuclear proliferation, Carter has repeatedly called for a worldwide moratorium on the sale of reprocessing and uranium enrichment plants, and for stringent controls on the export of other nuclear technologies. He has also proposed that an international conference be held to discuss alternative energy sources.

Perhaps the most important nuclear policy question confronting Carter, however, will be whether to permit spent nuclear fuel from commercial power plants to be reprocessed, and the plutonium extracted from it to be recycled as reactor fuel. Just five days before the election, Mr Ford announced that his Administration would defer a decision on reprocessing and recycling plutonium at least until the matter has been given more study. Many nuclear critics, including Carter, called the announcement "too little and too late", but Carter's own policies for

recycling have yet to be specifically stated.

Finally, Carter has promised to scale down the effort to develop a liquid metal fast breeder reactor (LMFBR), now the largest single federal energy programme. He has stated that he will reduce the effort to a relatively minor level and, perhaps, seek international participation in it. If Carter decides to

reject plutonium recycling and to scale down the LMFBR programme, those actions would constitute major defeats for the long-term plans of the nuclear industry.

● **Biomedical research.** Virtually nothing has been said during the campaign about the federal government's

Six-state nuclear battle: results

ALTHOUGH the nuclear industry in the United States is not too happy about the fact that last week's election will put into the White House a man who is decidedly cool toward nuclear power, it can at least take considerable comfort from balloting in six states. Voters in Arizona, Colorado, Montana, Ohio, Oregon and Washington soundly rejected propositions which would have placed crippling restrictions on nuclear plants in those states. A similar proposition was defeated in a seventh state, California, last June.

The propositions were rejected so decisively—by margins ranging from 58–42% in Oregon to 71–29% in Colorado—that the results constitute a stunning setback for the anti-nuclear movement in the United States. Spokesmen for the nuclear industry in fact wasted no time in claiming the outcome as a popular endorsement of nuclear energy. Carl Walske, president of the Atomic Industrial Forum, said last week, for example, that nuclear power "has been taken to the Village Square, as Einstein predicted, and has been approved by the American voter".

The defeats were particularly striking since opinion polls conducted only a month before the election indicated that the propositions stood a good chance of being approved in most of the states. In Colorado and Oregon, they were even leading by a margin of two to one. Anti-nuclear groups blame their defeat on the lavish funding poured into the campaigns during the closing stages by industry and labour groups anxious to swing voter sympathies against the propositions. Pro-nuclear forces are reckoned to have outspent their opponents by about 8 to 1, and it is noteworthy that in Montana industry groups put up more than \$80,000 to defeat an anti-nuclear proposition, even though Montana has no nuclear plants and no plans to build any.

The propositions would have forced new nuclear plants to meet three tough requirements before being put into operation. First, there

must be no limit to the damages which could be claimed by victims of a nuclear accident — at present Federal law limits the total liability per accident to \$560 million. Second, there must be an acceptable, proven technology for getting rid of nuclear wastes. And third, key power plant safety systems must be found to work satisfactorily. The last two conditions would have to be met to the satisfaction of at least two-thirds of the members of state legislatures. Those requirements are so tough that if they were passed in any state they would have virtually halted nuclear expansion in that state. Consequently, the industry fought them on the grounds that they would increase energy prices, discourage industrial expansion and increase unemployment.

The crushing defeat of the propositions in all seven states could have important national implications. For one thing it is likely to take the steam out of attempts in Congress to curb the growth of nuclear power. In the past few years, several bills aimed at halting nuclear expansion at least until a variety of conditions are met have been introduced. Though they have never enjoyed much Congressional support, if voters had demonstrated their enthusiasm for such measures by passing some of the propositions, anti-nuclear forces in Congress would have been given considerable impetus.

The outcome of the referenda is also likely to discourage similar attempts to limit nuclear power through direct ballot in the future. In fact, some nuclear spokesmen are already claiming that the crushing defeat of the propositions will lead to a sharp decline in anti-nuclear sentiment in the United States.

Although such claims will almost certainly prove to be exaggerated, the outcome of the referenda has clearly provided the nuclear industry with considerable ammunition to combat President-elect Carter's reservations about expanding the use of nuclear power in the United States.

\$2,000 million biomedical research programme, but again, Carter will be faced with some controversial decisions.

First, however, it should be noted that Carter's election may help reduce one of the chief problems which has plagued the federal biomedical research effort for the past few years. Major tussles between Congress and the Ford and Nixon Administrations over the size of the budget for the Department of Health Education and Welfare, of which NIH is a part, have frequently delayed the release of funds for NIH and thrown planning into confusion. The Carter Administration is likely to work more closely with Congress on such matters, and thus NIH's planning should be made easier.

Among the more touchy issues which Carter must sort out are whether to continue to support the training of biomedical researchers—Nixon and, to a lesser extent, Ford tried to phase out the NIH training programmes over noisy opposition from the scientific community. He will also have to adjudicate the growing controversy over whether the National Cancer Institute should continue to enjoy a privileged

position in NIH. In that regard, it may be recalled that Ford took a tentative step by suggesting that the cancer budget should be held constant this year while support for research on other diseases should be allowed to grow, but Congress increased the cancer budget anyway.

● **Arms control.** Though many of Carter's foreign policy statements have a distinctly hawkish ring about them, he has also criticised some arms control treaties as being inadequate. In particular, he has called for a complete cessation of nuclear testing, and he has labelled the proposed Threshold Test Ban Treaty as "totally inadequate". That treaty is now pending before the Senate for ratification, and it is likely that Carter will try to negotiate something more substantial instead of trying to get the measure through the Senate.

● **Congress.** Several key Senators and Congressmen who have been influential in science policy affairs have either retired or were defeated in the polls last week. The chief casualty was Senator Frank Moss, chairman of the Senate

Committee on Aeronautical and Space Sciences. But perhaps the most important changes are likely to come in the powerful Joint Committee on Atomic Energy, where the chairman, John Pastore, and Stuart Symington have both retired, and Senators Joseph Montoya and John Tunney were both defeated. On the House side, James Symington, the chairman of the subcommittee which oversees the work of the National Science Foundation, relinquished his House seat to seek election to the Senate. He has been an important defender of NSF during the past couple of years, but at least his loss will be partially balanced by the fact that John Conlan, NSF's chief critic, also relinquished his House seat to run unsuccessfully for the Senate.

Though many important science policy issues have been virtually ignored during the election campaign, Carter's handling of them in the next few months will be closely watched. Leaders of the scientific community are not usually reticent in giving policy advice to the government, and they can be expected to make themselves heard in the coming weeks. □

AUSTRIA

Nuclear consultation

Engelbert Broda writes from Vienna on Austria's nuclear energy information campaign.

UNPRECEDENTED action has been taken by the government of the Republic of Austria. In view of the wide divergence of opinions on the desirability or otherwise of nuclear energy and the emotions aroused, it has decided to consult the population on the matter directly. Although decisions will eventually have to be taken by Parliament, the government is making sure that the views of the people can serve as a basis. A nation-wide information and discussion campaign has been organised, financed by the office of the Prime Minister, Bruno Kreisky, in collaboration with the "Energy Section" of the Federal Ministry of Trade and Industry. The section is headed by Wilhelm Frank, a man with wide scientific interests who is not only a professional mechanical engineer but also a renowned theoretical mathematician and a philosopher.

Austria's energy position is relatively favourable. While for many years power consumption increased by about 7% per year, the rate of increase has, as elsewhere, gone down more recently. Hydroelectricity still accounts for more

than 70% of total electric power, and there are still unused reserves of hydropower, mainly along the Danube. (It is interesting, though, that a popular movement for the protection of the Wachau, the part of the Danube valley with the most beautiful scenery, has induced the government to shelve and reconsider plans for the exploitation of that stretch.)

The production of brown coal, mainly in Styria, is considerable and goes mostly to power plants near the pits. While normally some electric power is exported, in emergencies power can also be imported by high voltage transmission lines from west and east. About one-fifth of the oil and gas used in the country comes from wells in Austria. The necessary imports are widely spread geographically: sources include the Middle East and Russia. One nuclear plant, a 700 MW boiling water reactor on the Danube about 40 km north-west of Vienna, is in an advanced stage of construction.

That the first nuclear power station will be taken into exploitation is hardy in doubt. But although detailed projects exist for further stations, no decisions have been taken as yet. At a press conference held soon after the elections in Sweden that were so influenced by the nuclear issue, Austria's



Austria's Chancellor, Bruno Kreisky

Federal Chancellor cheerfully said that he was doubtful about the wisdom of more nuclear power, while the Minister of Trade, Herr Staribacher, expressed himself in favour of additional stations. In such circumstances the voice of ordinary people will clearly have a lot of weight.

The Federal Press Service has issued a little book containing basic information, as objective as possible, on nuclear power. There will be 10 public meetings in various towns where the members of the public may ask questions and offer comment for up to 90 seconds each. Each meeting deals with a different aspect of nuclear energy—

socio-economic problems, energy policy, safety, social repercussions, influence on climate and biomedical problems. The discussion on each subject can be held in one place only, but written contributions are also accepted.

Each meeting is prepared by a subgroup of five or six experts, including natural scientists, sociologists and economists. West Germans, Swiss and even an eminent Bulgarian are among the members. In preparatory meetings, each group tries to reach factual agreement as far as possible but does not hide internal divergences when it faces the meeting. Disagreements are bound to be pronounced as in every case both friends and foes of nuclear energy have been appointed to the groups. They do

not include, however, members of the nuclear establishment, notably employees of the companies that build or plan nuclear power stations.

The first public meeting was extremely lively. Following the meetings each group will submit an extensive report which will be further discussed publicly between adversaries and supporters of nuclear power. No party whip will be applied in Parliament, the Chancellor has said.

If in the end a major expansion of nuclear power production were rejected, Austria would have to take drastic steps to curb the further increase in power, and energy consumption generally. Alternative sources of energy, save undesirable imports, are in

the short term limited though the long term prospects for solar energy seem good. There is no doubt, however, that the scope for energy conservation in Austria is enormous: energy has been anomalously cheap, with oil prices low until 1974 and the sales price of electric power not keeping pace with inflation.

One perhaps inevitable, if regrettable, feature of the information campaign is the near-absence of a crucial issue: the subject of the plutonium economy and weapons proliferation. This problem is not so obvious to the citizens of a small and neutral country that spends little on the military; the success or otherwise of the bold venture they have embarked upon, however, will be watched closely. □

REPROCESSING

Permission to process

Gillian Boucher reports on recent developments in plans for the reprocessing of nuclear fuel in Britain.

THE decision last week by Cumbria County Council's Planning Committee to approve in principle the £600-700 million programme for the expansion of the nuclear fuel reprocessing plant at Windscale brings Britain perceptibly closer to becoming a world reprocessing centre. Unless Mr Shore, the Secretary of State for the Environment, exercises his option to re-examine the question within 21 days of receiving notification of the committee decision, outline planning approval is automatic. That will remove probably the greatest single impediment to the signing of the controversial nuclear reprocessing deal with Japan.

To gain planning permission will be an important victory for the nuclear industry which, since the negotiations with Japan were publicised at the end of last year, has been fighting a noisy battle with well-organised environmental groups. Fears centre on the prospect of Britain taking on long-term storage of other countries' fuels which she has reprocessed—becoming what the popular press has called the world's nuclear "dustbin"—and the dangers of sabotage if plutonium is shipped back to the country of origin.

After a five-month delay to the negotiations with Japan to allow time for public debate, Mr Anthony Wedgwood Benn, the Secretary of State for Energy, announced in March of this year that foreign deals would be acceptable if Britain did not have to store the fuels on a long-term basis but had the option of returning them

to the country of origin. But the Flowers Commission said in its report on nuclear power and the environment that it would be unwise to take up the option, that it was best to avoid transporting the wastes if possible and that long-term storage in geological formations would almost certainly be safer in the UK than in seismically active Japan.

The deal originally being discussed with the Japanese utilities was for British Nuclear Fuels Ltd (BNFL), the state-owned company which operates Windscale, to reprocess 3270 tonnes of spent Japanese oxide fuel during the 1980s. The deal was worth around £4000 million including a large

down-payment. But the delay for public debate gave the French reprocessing organisation, Cogema, a chance to declare its interest; both BNFL and Cogema, a subsidiary of the French Atomic Energy Commission, are partners in United Reprocessors, an Anglo-French-German market-sharing body. The final contract, now likely to be signed in the next few weeks, will probably share the Japanese work equally between Britain and France. British contracts with other countries are also being negotiated.

BNFL has had government approval of its plans to invest £245 million on extending and improving the existing Magnox fuel reprocessing plant and £40 million on development of the vitrification process; it still awaits the crucial approval of the £350 million



Windscale reprocessing plant

investment on Thorp, the oxide fuel plant, which will be vital if Britain is to become a big exporter of its reprocessing services. If business prospects continue to look good, permission will almost certainly be forthcoming.

Cumbria County Council has had a daunting task in assessing the application for planning permission—weighing on the one hand 2,000 jobs in an area of high unemployment and the prospect of much-needed foreign earnings, some of which have already been lost to France through delays, against unfamiliar dangers to a rural community on the other. The Windscale workers have been staunchly in favour of expansion and wanted the County Council to make its decision without further delay. But Friends of the Earth, Half-life, the Conservation Society and the Town and Country Planning Association among others, concerned principally with the oxide plant, wanted the matter referred to a public enquiry.

In the end the committee passed a resolution saying that it was "minded to approve" the application "subject to agreement of appropriate conditions" but passed the final responsibility over to Mr Shore as the application was a "departure from a fundamental provision of the county development plan". At the beginning of this week the Department of the Environment said it had still not received formal notification of the Committee's decision. The pressure groups will continue to call for a public inquiry in the little time remaining.

BNFL has been accused by the Lawyer's Ecology Group of providing

Why reprocess?

THE short answer is to make plutonium for fast breeder reactors. Though fast breeders will make us virtually independent of U_{235} sources, the supplies of plutonium and depleted uranium produced by the present generation of thermal reactors are essential for them. But much depleted uranium is treated before use in thermal reactors. Reprocessing also makes environmental sense. The 30% waste left after the removal of uranium and plutonium is more compact and easily stored than the untreated spent fuel.

A reprocessing works is essentially a straightforward chemical plant with elaborations necessitated by the radioactivity of the materials. The burnt fuel (uranium metal in the case of the Magnox reactors currently in use in Britain, uranium oxide for other reactors including AGRs and PWRs) is dissolved in nitric acid and the uranium and plutonium extracted with an organic solvent. The remaining highly active acid solution containing a variety of α and β emitters is at present stored in elaborate stainless steel tanks.

Incorporating the wastes in a solid would allow less hazardous storage, perhaps in deep boreholes either on land or under the sea. Solidification

also makes long-distance transport feasible and, if transport of wastes back to their country of origin is made compulsory, will be the *sine qua non* for Britain's role as a major international processor of nuclear fuels. A process of vitrification developed at Harwell in the late 1950s and known as Harvest has been taken up by British Nuclear Fuels who hope to be constructing a demonstration plant at Windscale by 1980. But the technological prospect is daunting, combining as it does intense radioactivity with the high temperatures needed for glass making.

Another large question mark hangs over the business of processing oxide fuel which, being more highly irradiated, poses greater problems than Magnox fuel. At present there is no commercial scale oxide fuel reprocessing plant in operation anywhere in the western world, though the La Hague plant opened earlier this year is approaching that level. At Windscale a "head-on" plant for pre-processing oxide fuel before it joined the Magnox fuel was in operation, but an accident in 1973 in which a number of workers were contaminated led to the closure of the plant. It is expected to reopen next year.

the County Council with "the sketchiest ever (application) ever offered for any major development" and of leaving it "until all the options were closed". But although the replies to the County Council's detailed questions were only

just ready in time for the public debate, Professor J. H. Fremlin, the County Council's independent scientific consultant, found he had all the information he needed to assess the dangers to the public at large. □

BRITAIN

A case of suitable treatment?

A number of members of the World Federation of Scientific Workers received inexplicable treatment from the Home Office when they tried to enter Britain for the federation's 11th General Assembly on September 17-23. Professor Eric Burhop of University College London, who is President of the federation, gave Nature his view of what happened

THE Federation's General Assembly, its highest policy-determining body, meets every three years. This year, on the occasion of its 30th anniversary and amidst some difficulty for many participants, the assembly was held in London, where the organisation was founded. Professor Burhop tells his story with the aim of preventing another recurrence in the future—the

assembly had once experienced even more serious difficulties some 15 years ago.

Before the latest meeting, says Burhop, a British MP associated with the affiliated organisation ASTMS explained to the then Home Secretary, Mr Roy Jenkins, the nature of the planned meeting and the countries from which the attending scientists would come. That was in February. Mr Jenkins' reply, according to Burhop, stated that he could see no reason why the assembly should not meet in London; but he added that people coming from countries from which visas were required should make their application in good time and certainly not less than two weeks before they wished to enter Britain, to give time for the necessary processing. "We informed our affiliated organisations

accordingly," says Burhop, "and expected there would be no more trouble. It was not until September 14, three days before the Assembly was due to commence, that we began to receive cables from delegates from distant parts that their visas had not been issued."

Burhop is quick to repudiate the frequent charge that the federation is a communist-controlled or Soviet-dominated organisation. That is not true, he says, and never has been. He summarises the aims of the federation as international scientific cooperation, the protection of the freedom and rights of scientific workers, the constructive application of science and international agreement on disarmament and the eventual abolition of nuclear weapons. He describes the attempts of the federation after the Second World War to hold meetings in London to discuss nuclear weapons as "sufficient to send sections of our

military and political establishment into a flat spin".

Official attitudes appear to have relaxed in recent years, but the federation's latest troubles, as told by Burhop, suggest that it is still viewed with deep suspicion. First to complain of difficulties was an Egyptian professor who had applied in Cairo on July 25 for his visa. Burhop says he received the last of several telegrams from the professor on September 16, saying that owing to the non-arrival of his visa he had cancelled his flight. The professor's visa was finally granted three hours before the commencement of the first meeting he was due to attend. A professor from Vietnam was the next in difficulties. He had applied for his visa five weeks previously at Hanoi, and it was granted on September 16—too late to catch the weekly direct flight from Hanoi to London.

Both professors had visited Britain previously without any difficulty, the Egyptian on many occasions. They were each able to reach London by circuitous routes, but some two days late. Four scientists who formed the delegation of the Organisation of Korean scientists in Japan were not so fortunate. They were members of the community of North Koreans who were stranded in Japan after the Second World War and not allowed to return to North Korea; unlike Japanese nationals, they needed visas to enter the UK, and re-entry permits to return to Japan. The Home Office gave the date of receipt of their visa application as September 2, which allowed the required two weeks for processing; the scientists claim that they applied even earlier. Their visas were granted on September 22, the day before the assembly ended.

But as Burhop points out, Home Office procedures were shown in the oddest light in the handling of the Soviet applications. After the French delegation, the Soviet delegation was the second largest at the Assembly. It consisted of twelve persons, including two Academicians, one a Nobel laureate and another a famous mechanical engineer, a James Watt medalist of the British Institution of Mechanical Engineers. Both members of the Supreme Soviet of the USSR (one is on its Presidium), they had visited Britain many times.

Burhop takes up the story: "There is a discrepancy in dates when the applications are said to have been made. Our Soviet colleagues claim that their Ministry of Foreign Affairs transmitted all the applications together to our Embassy in Moscow on September 3, exactly two weeks before the date of travel as specified by Mr Jenkins. The Home Office claims that they were received by our Embassy only on

September 8. Late on September 17 I was informed by the Home Office that three of the Soviet visas had been granted, the rest were under consideration, and no more would be granted until Monday. Of the three visas granted two were of secretaries to the delegation. The other was a social scientist and authority on disarmament. The Soviet delegation that sets great store on its distinguished Academicians was deeply offended. It read into the decision a studied insult, which I do not believe was intended, and I feared at one time it might develop into a diplomatic incident. The remaining Soviet visas, with one exception, were granted about midday on September 20, in time to permit the whole delegation with the exception of the scientist whose visa was refused to travel to London that evening. They were able to make an effective contribution to the rest of the Assembly."

Then there was the GDR delegation, which included the President of the GDR Academy of Sciences and a university rector. Their application was late, and was received only on September 10. The Polish delegation, however, applied at the same time and received visas almost on time. Visas for the whole of the GDR delegation were granted only on September 21. They arrived the following day and participated only in the tail-end of the assembly.

Burhop again takes up the story: "Of course I do not excuse those delegations who applied late. But some of those who applied very early were also very late in receiving their visas. I was informed by the person handling the visas at the Immigration Department that all the visa applications had arrived on his desk only on September 15 and only then could they begin the usual processing. This was despite the fact that as early as July our office in London had started supplying to the Home Office lists of all the delegates coming from abroad, details concerning whether they needed visas or not and, by early September, the name and country of each participant."

What went wrong? The change of Home Secretary on September 15 may have caused difficulties. The new Home Secretary, Mr Merlyn Rees, was absent from London on September 17 and September 20; it may be that the civil servants whose responsibility it was to take the decision were not willing to do so in the absence of the new minister. But Professor Burhop feels that no matter when the visas were applied for it made no difference: processing of them only started two days before the assembly was due to start and proceeded at a leisurely pace.

The most curious case of all is that of the Soviet delegate, Professor

Grigori Kotowski, whose visa was refused. One newspaper telephoned the Home Office and was told that Professor Kotowski was only an organiser, not a scientist at all. But according to Professor Burhop and others he is a social scientist of great eminence, deputy head of the Institute of the Peoples of Asia of the USSR Academy of Science and a leading authority on India. Kotowski has frequently visited the UK without any trouble, only last year attending on behalf of the USSR an executive meeting of the International Economic History Association.

Burhop speculates that the refusal of his visa was conceivably a matter of bureaucratic face-saving. Having boomed about the issue of visas the Home Office felt it had to refuse one to give the impression that there were very serious reasons for the delay. Alternatively—and less flattering to the Home Office—Burhop suggests a mix-up. "The name Grigori Kotowski is very well known in the Soviet Union. It is that of an almost legendary hero, a partisan leader, famed for his military exploits in Moldavia during the Civil War. There is an imposing statue to him in Kishinev. A tank regiment in the Red Army is named after him.

"Professor Grigori Kotowski is his son. A less military looking man or a man more academic or professorial in bearing than Professor Kotowski it would be impossible to imagine. But dare I suggest that the Home Office have got the records mixed up and that when they refused a visa to Professor Grigori Kotowski they mistook him for his father? Of course there is a small matter of fifty years between the careers of the two Kotowskis. Nevertheless in all this matter the Home Office has seemed so maladroit that nothing would surprise me."

The Home Office itself, when contacted by *Nature*, was hardly illuminating. The applications from North Korea, it admitted, did take a "little bit longer" than others. "We're sorry about it", a spokesman said, but "it took this time to clear. We take our responsibilities very seriously". Asked about the case of Kotowski, he explained that reasons were not given for the refusal of a visa; it depended a lot "on the relationships between one country and another". But he made a suggestion: why didn't *Nature* give the number of people who did receive their visas on time?

The response is printable. Of the 100 or so people attending, 41 required visas to enter Britain: 16 received their visas in time, 20 received them late and so missed at least 2 days of the assembly, 4 received visas too late even to attend, and one had his visa refused.

□

SCANDINAVIA

Controlling the pollution trade

Under a unique treaty which entered into force last month, private citizens living in the Nordic area will have the right to complain about and be compensated for damage to their environment caused by activities in their neighbouring countries. Mike Duckenfield reports

THAT the first agreement of its type should be reached between the Nordic nations is no surprise. Norway, in particular, suffers badly in the present European exchange of pollution and the Scandinavians as a whole are keen to secure international consensus on its control. In deciding to put their own house in order they are not only showing their seriousness, but aiming to preempt any criticisms about their own lack of action.

The agreement, prompted by the Nordic Council and covering all five of its members except Iceland, will be supplemented next January by Norwegian legislation requiring industry to use low-sulphur fuels. In October next year a start is due to be made to a seven-year Swedish plan to cut sulphur emissions to their level in the early 1950s.

The Swedish plan follows a recent government commission appointed by the Minister of Agriculture which carried out an extensive survey of air pollution problems caused by the burning of fossil fuels containing sulphur. It estimated that in 1973 about 60 million tons of sulphuric fallout, either dry or in precipitation, was generated in Europe, compared with only 25 million tons between 1910 and 1950. Of this, 38 million tons came from Eastern Europe and the remainder from the West. In addition, increased energy consumption in all countries was expected to result in the generation of 74 million tons by 1985, with 27 millions tons coming from the West.

In 1974, Sweden itself burned about 19 million tons of fuel oil, two-thirds of it heavy. As oil with a sulphur content of 2.5% by weight causes emissions of about 50 kg of sulphur for every ton of oil burned, it was calculated that about 800,000 tons of sulphur was emitted, a quarter of it from industrial processes using sulphuric compounds and the remainder from burning oil. If no action was taken this would rise to one million tons by 1985. As it is, the aim is to cut it to 300,000 tons.

To achieve this the Swedish plan involves banning the import and sale of light fuel with more than 0.5% sulphur

nationally from next October and, at the same time, prohibiting the use of heavy oil with more than 1% sulphur in the part of the country south of a line north-west of Stockholm. Two years later, the ban would extend to light oils with more than 0.3% sulphur and, in 1981, the extension nationally of the heavy oil ban. The plan also requires that the existing ban on burning oil with more than 2.5% sulphur be placed on other fossil fuels, most notably coal.

The programme would cost an estimated 500 million Swedish crowns (about £72 million) and includes government grants to finance the experimental liming of some of the 10,000 or so lakes that have been acidified by sulphuric fallout. At present it costs between Skr 400 and Skr 500 per hectare to lime the lakes and the job has to be repeated every five years.

For all this, Sweden expects to be able to do no more than maintain the *status quo* in the worst affected southern and western parts of the country. The reason is simply that most of the pollution is imported, mostly from the south and west across the North Sea and Baltic. In these areas it is estimated that less than 25 per cent of the fallout is Swedish in origin. In the remainder of the country the figure varies from between a quarter to a half of the total. Nationally it is just short of one-third. Because of this, the Swedes would now like to see total emissions in Western Europe brought down to between six and eight million tons within the next 10 years, a task that would cost about Skr 20,000 million a year.

In neighbouring Norway the situation is even worse with less than one-tenth of fallout being home-produced. Even in the area around Oslo, the country's most industrialised region, the figure only reaches 30-40%. The thin soil over much of the country does little to neutralise the acid rain and the build up of polluted winter snow leads to an annual mass slaughter of thousands of hatching and spawning fish when the spring thaw pours into the rivers and lakes. In the worst-hit south-west salmon and trout have disappeared from many rivers and a recent survey of 153 previously well-stocked lakes revealed the eradication of all types of fish from all but two of them.

So serious has the problem become that the Norwegian government is currently spending about £6.5 million in employing 60 full-time scientists on a seven-year programme to examine the effects of acid precipitation. And to draw international attention to the

need for control, the Norwegian Ministry for the Environment together with the precipitation project (SNSF project) this summer invited representatives from 20 nations including all but Luxembourg of the Common Market countries, the Soviet Union and three Eastern European nations to a conference at Gaustadblikk near Rjukan in Telemark in the south-west.

Research shows that not only are fish being killed, but acid rain is affecting soil productivity and could be causing decreased forest production due to the depletion of the earth's nutrients. Similarly, the Swedish commission report also points to the effects on urban areas due to the corrosion of metals and stone, especially limestone and marble. Costs of quicker replacement of materials and the need for corrosion protection were put at anywhere between Skr 100 million and Skr 1,000 million annually, not including the irreplaceable damage done to historic buildings and monuments.

While the conference could only agree to recommend nations to reconsider their approaches to emission control and to sponsor more research, international cooperation may be given a much needed boost with the publication of a report by the Organisation for Economic Co-operation and Development next year. OECD's recently completed research project into the long-range transport of air pollutants will show just how badly affected southern Scandinavia is. In one extreme case in January 1974, winds from all over Europe brought 4,000 tons of sulphate and dumped it over about 20,000 square km of southern Norway in just 12 days. Some areas had nearly a ton of sulphate per square kilometre. □



Pollution demo, Swedish-style

IN BRIEF

Appointment of GMAG chairman

The first chairman of the British Genetic Manipulation Advisory Group is to be Sir Gordon Wolstenholme, Director and Secretary to the Trustees of the Ciba Foundation and President of the Royal Society of Medicine. The establishment of a group to advise laboratories on appropriate precautions in genetic experiments was the main recommendation of the Williams working party which reported in August this year. The group will examine proposals from laboratories (though precisely which types of experiment will have to be submitted is yet to be decided), assess the hazards involved and advise on appropriate precautions. The names of other members of the group will be announced soon.

Customers for nuclear club?

The secret club of 13 nuclear exporters, which aims to restrict nuclear exports to countries which have not ratified the 1970 Non-Proliferation Treaty, is reported to be considering admitting customer nations to its circle. The group is expected to meet in London this week. Customer nations made their resentment of the group's clandestine activities clear at the International Atomic Energy Agency annual conference in September; it has been suggested previously that the group admit customer nations but some members, notably France, were against it. Iran is being suggested as a possible first customer-member, as its large nuclear programme involves exports from several countries including the United States, Germany, France and the United Kingdom.

French chemical controls

Production and import of chemicals in France will be much more strictly controlled in future if a draft law adopted by the Council of Ministers last week is passed in Parliament. Producers and importers will be required to make a study of the effects of new chemicals on man and the environment and make their results available to the authorities, who will have six months to decide whether the manufacture, sale or use of the chemical should be subject to regulations or even banned completely.

The measure will fill a gap in existing legislation, which regulates the use of specific products such as explosives, pesticides and food additives and protects certain defined groups. The new law will be the first of its kind in Europe.

BRAZIL is a country of extremes; wealth and poverty, grandeur and squalor, but above all, tremendous vitality and optimism. What other country could build such a fantastic new capital as Brasilia? Brazil is completely fascinating to a biologist, whether standing on a street corner in downtown Sao Paulo and watching the complete ethnic diversity of the hurrying crowds, or gazing from a plane at the unbroken green carpet of wild vegetation stretching for hundreds of miles below, without a sign of human impact. Or seeing the "mud house" nests of a charming bird, Joao de Barro, on the crossbars of most of the power line poles along the highway going south from Porto Alegre.

Brazil is where the high technology of the 1970s is brought to bear on one of the last great untouched areas of the earth. Can we hope that enlightenment will come in time to enable this country to escape the rapacity of human beings and their new machines? Sometimes, the results of change are obviously beneficial. Large numbers of Japanese immigrants have come to Brazil in recent years. The farmers among them were accustomed to producing large yields from their tiny farms in Japan. They found that land was plentiful in Brazil, and they evidently apply the intensive methods they used in Japan to much larger farms. (Brazil is the world's fourth largest market for pesticides.) The result is that the huge city of Sao Paulo now receives a plentiful supply of splendid fruits and vegetables, probably for the first time.

I saw these commodities exchange-

ing hands in the big wholesale markets at midnight. The produce was as carefully packed and graded as if it had been intended for gift shops. The nutritional status of many Brazilians must have been greatly improved by the industry of these new farmers. But in some regions, malnutrition is

Brazilian dilemma



THOMAS H. JUKES

common. When I visited the Ministry of Health in Brasilia, I was told that vitamin A deficiency is so prevalent in north-eastern Brazil that the changes produced by it obscure the "Pap test" in women. Also, the deficiency causes blindness in many children. For people who cannot or will not eat green leaves, synthetic vitamin A should be added to food. It costs about three cents per million units—about \$150 for one million adult Recommended Daily Allow-

ances or for two million for children under four.

The huge Volkswagen factory at the western edge of Sao Paulo has helped to produce an "instant middle class" in Brazil. On city streets, drivers blow their horns and flash their lights at each other, to challenge the right of way, except when, as often happens, traffic is completely clogged.

A great question for the future of the biosphere concerns the fate of Amazonia, which includes six states and portions of three more. The Companhia Industrial de Amazonia was formed in 1966 for producing non-ferrous metals; Sudam is the agency for development of Amazonia including plans for iron ore and bauxite, for hydroelectric power, timber-cutting and raising cattle.

Twenty per cent. of the world's fresh water supply flows through the Amazon basin network of rivers. Amazonia's area is nine times that of France. Its colossal jungles have a canopy of green vegetation above a thin layer of fragile soil and resting on hardpan. When the jungle is cut down the topsoil tends to disappear, leaving an infertile base. It seems that little is gained by destroying the jungle, and at stake is one of the world's great photosynthetic factories for maintaining atmospheric oxygen. The birth-rate of Brazil, where Roman Catholicism is the state religion and divorce is not permitted, results in a population growth of about 2.8% annually.

Will a desire to preserve Amazonia override the pressures for Brazil's western expansion?

correspondence

Biological fly killer

SIR,—K. M. S. Aziz (October 14, page 544) misquotes F. E. G. Cox (August 19, page 646) when referring to the use of insecticides for the eradication of tsetse flies (principally *Glossina morsitans*) from some 27,500 km² of riverine vegetation in Nigeria. This was achieved by ground spray techniques and not by aerial spraying.

This is a point of detail and Aziz could well reply that it does not affect his argument that aerial spraying (as practised against *Glossina*, elsewhere in Nigeria and in other African countries) is an unsatisfactory method of controlling insect species. He gives as an example the resurgence of mosquitoes after an aerial spray operation over parts of Bangladesh. However, mosquitoes in Bangladesh present different problems to tsetse flies in Nigeria and it can be misleading to compare the two situations.

I suspect that the mosquitoes of Bangladesh re-appeared because the aerial spraying operations had no permanent effect on their habitats. Precisely the same would almost certainly have happened in time in the case of the tsetse flies of Nigeria had the habitat remained unaltered. However, Nigeria is a land-hungry country and the success of the anti-tsetse operations allowed a breathing space in which land cleared of *Glossina* was taken over by man and his cattle, altering the vegetation to such an extent that it no longer presented a suitable habitat for *Glossina*.

The moral of the Nigerian success story is that tsetse control operations should not be initiated unless there is a demand for the land occupied by the flies. Obviously, in order to prevent such evils as overstocking and soil erosion, exploitation of cleared areas must be strictly governed by well thought out development programmes.

Insecticides for tsetse control do have, and will continue to have for the foreseeable future, an important part to play in the development of rural Africa. By all means let us undertake research, as advocated by Aziz, on more specific biological methods of control; but Africa cannot afford to postpone action against *Glossina* until satisfactory alternatives to insecticides have been developed.

Your faithfully,

A. M. JORDAN

Tsetse Research Laboratory,
Langford, Bristol, UK

Nobel prizes

SIR,—I have found what I believe to be a significant correlation between Nobel prizes and Olympic games. Previously, the United States did not have the dominant position in science that it has achieved today. The Nobel prizes of physics, chemistry and medicine were never awarded to US scientists in the same year; now the situation has changed. In 1968 all three prizes were for the first time awarded to American scientists. The second favourable conjunction came in 1972 (seven titles out of eight, in the three domains). Now in 1976 there is again an American monopoly. The three remarkable events are significantly correlated in time with the Olympic Games.

Some friends of mine suggest that the

truly significant correlation is to be made with the presidential elections in the United States. Such a hypothesis is easily ruled out on the basis of the authoritative works of Einstein and Garfield. It is known that the presidential elections occur after, not before, the designation of the Nobel laureates. Einstein's theory of relativity (*Ann. d. Phys.*, 17, 891; 1905) excludes any time-reversed determinism. Then, the objectivity and political independence of Stockholm's academicians is attested by the fact that the Nobel prize correlates very well with the value of the distinguished scientific work, as measured in the Science Citation Index (Garfield, *Nature*, 242, 485; 1973).

Yours faithfully,

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Stamps of scientific interest

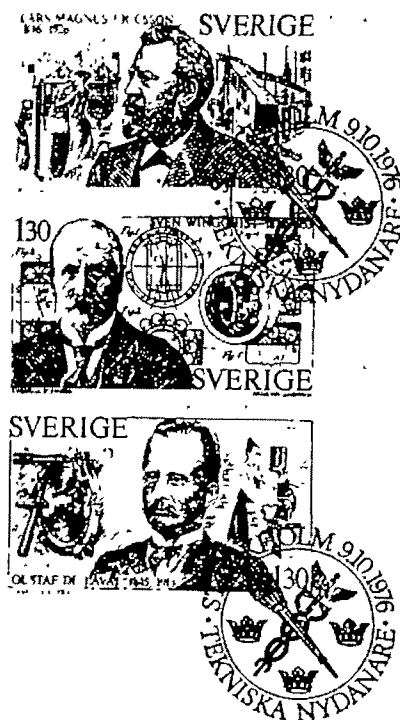
On October 9 Sweden issued a colourful set of five stamps commemorating nineteenth century Swedish pioneers in various spheres of technology. Each of the stamps bears a portrait of the pioneer in question together with an example of the practical application of his contribution to technology.

These range from work on the use of solar energy (John Ericsson), a machine gun and hay-mower (Helge Palmcrantz), the first desk telephone (Lars Magnus Ericsson), a milk separator (Gustav de Laval) to the first spherical, self-regulating ball bearing (Sven Wingquist). It was this latter innovation which was later produced by the Swedish Ball Bearing Company (SKF), in Gothenburg, which was founded in 1907. L. M. Ericsson's first desk telephone of 1877 was the start of the L. M. Ericsson Telephone Company, now a world-wide enterprise.

Also issued by Sweden on October 9 were two stamps devoted to industrial safety. The first law on this subject in Sweden was passed in 1889, it also being then that the first inspectors were appointed. The present body both investigates hazards on the factory floor and carries out research on occupational health. The design of these two stamps consists

of two hands against a background of two cog wheels. The fingers and the cogs join to symbolize the interplay between man and machine.

Ian F. Finlay



news and views

Joint Oceanographic Assembly

from C. M. Yonge

The 4th Joint Oceanographic Assembly was held in Edinburgh on September 13-24. Thirteen organisations participated under the auspices of a group of international bodies led by the Scientific Committee on Oceanic Research of the International Council of Scientific Unions.

WHAT immediately emerges from these meetings is the enormous recent extension of knowledge in effectively all aspects of marine science. This comes very forcibly to one who worked at the Plymouth Laboratory when W. R. G. Atkins and H. W. Harvey were elaborating the first colorimetric methods for the quantitative determination of phosphates and nitrates which led to elucidation of the annual cycle of events in surface waters, and who first visited the now mighty Scripps Institution when this consisted of a single laboratory, pier and a few scattered staff houses!

Geology which was then of minor concern has since assumed supreme importance. The first impact of plate tectonics is now past. With the accompanying study of sediments, now cored in supreme depths by the *Glomar Challenger*, aided by isotope detection and carbon dating, it provides evidence for the history of the oceans which was inconceivable a generation ago.

The oceans have long been divisible into great latitudinal zones with surface cyclonic or anticyclonic circulations and underlying compensating currents, the deepest waters creeping slowly in an equatorial direction. Upwelling off continents and between diverging current systems appeared as the major means of surface enrichment. W. H. Munk (Scripps Institution, San Diego) now reveals that the greater part of oceanic movement is highly variable, that an a.c. rather than a d.c. circulation prevails. A series of casual currents produces eddies in midwaters. In the Atlantic, some appear to originate by the cutting off of marginal areas of the Gulf Stream but in

general their origins are obscure. Of their great significance there seems no question, absorbing, as Munk calculates, more than 90% of oceanic energy. The superficial zone of high productivity is revealed by infrared photographs taken from satellites. All previous estimates of marine productivity may need considerable modification.

Wartime studies associated with submarine detection revealed the ubiquity and variety of submarine noises, readily detectable because sound pressure in water is a thousand times greater than in air. As L. M. Brekhovskikh (Academy of Sciences of the USSR) reminded us, while we, on the surface, cannot hear the fish below, anything we say may be carried to them. Internal probing of the sea by high frequency ultrasound can provide an acoustic image where no optical image is possible. Meanwhile a widening series of surface measurements—of temperature, currents, wave heights, chlorophyll and sediment concentrations and of much else—become possible from the orbiting platforms of satellites.

The complexity of the marine environment and so of the biological processes within it was evident in many contributions. The greatest of fisheries is that for the anchovetta in the rich and cold upwelling waters off Peru. Productivity is irregularly affected by incursions of warmer waters, the dreaded 'El Niño', from the north. But what had appeared as a direct effect on the fish—and so on their original exploiters, the guano-producing boobies, cormorants and pelicans—now appears as much more complex. The actual nature of 'El Niño' is in question as reported by G. L. Kesteven and L. J. V. Gonzales (Instituto del Mar, Lima). The effect may be less on the adult fish than on their reproduction and on development and survival of young stages possibly predated by medusae and pelagic crabs. Already it seems that the relatively very low spawning stock of anchovies in 1975 has produced more abundant progeny than the more

numerous stocks in the sixties. Until the precise nature of controlling processes becomes clear there can be no certainty about future stocks.

The long term variability of conditions in the Baltic, as indicated by the known vagaries in the herring fishery since the Middle Ages, now fits within a more recently traced pattern of ocean change in the North Atlantic. Warming of these waters with accompanying northward extension of major stocks of food fishes, including development of a cod fishery off the west coast of Greenland, has been followed by recent cooling and withdrawal.

A discussion of conditions in the intensively studied North Sea by A. Lee (Fisheries Laboratory, Lowestoft) provided some insight into what can happen when a body of water is bordered by areas of intense industrial activity. Here the water is exploited for fish, the bottom deposits are a source of aggregates for use in the building industry and the deeper deposits contain large quantities of oil and gas. On the credit side, although domestic and industrial effluents enter in vast quantities, extensive testing has failed to indicate any serious accumulation of pollutants in coastal animals. In the North Sea the major risk comes from escape of oil, directly from the borehead or by fracture of a pipe line. In the open oceans there is a possible future risk when pollutants slowly building up in the profound depths regain the surface.

Removal of wide areas of gravel could certainly have devastating effects on bottom-living animals and especially on the deposited eggs of herrings. The closure of shallow water areas such as the Wash and the Dutch and German Waddensee—both possible—would destroy the nursery grounds of plaice and other food fishes.

The widely publicised destruction of herring stocks, due largely to their over-exploitation for industrial purposes, has strangely been accompanied by great increase in the stocks of mid-water and bottom-living round fishes, such as haddock, which also spawn a

year sooner than formerly, making nonsense of earlier predictions of future stocks. The phosphate content in the southern North Sea has actually doubled in the past 15 years. Here we encounter what must surely be a positive result of human interference. Opinions vary between explaining this as the result of increasing discharge of largely domestic effluents from the surrounding countries or as an indirect consequence of the destruction of the herring fisheries by way of a now much better nourished and more numerous bottom fauna.

These are but few of the topics that claimed attention in a programme which ranged from mathematical treatment of data concerning water movements to stratigraphy, and from man's use of oceanic lagoons to the possibilities of ocean engineering and of extracting energy from salinity differences.

One cannot but be impressed by the obviously increasing integration of marine studies which was reflected in the attitude of the participating scientists. Any venture on or in the high seas calls for international plan-

ning and the cooperation of research vessels from different nations, the greatest such achievement to date being the International Indian Ocean Expedition. It follows that assemblies such as this one are essential if scattered workers are to meet and plan common activities.

Yet this assembly met with a sense of impending difficulties or worse. The Law of the Sea Conference falters on but with the eventual possibility that the present freedom to conduct oceanic research may become hopelessly entangled with far extended and jealously guarded international boundaries. As the mineral resources of the land diminish, those of the sea, notably of the manganese nodules—with significant additions of other metals—so abundant in oceanic depths, become increasingly attractive. Here is an extreme case of "haves" and "have-nots" with only a handful of the wealthiest nations able to afford the equipment needed for oceanic research and so obtain information that could be withheld from others. Such problems may well come to preoccupy future assemblies. □

Anything new about reverse transcriptase?

from Karin Mölling

YES, indeed, but the news is somewhat embarrassing! Since the discovery of viral reverse transcriptase in 1970 the problem has been to transcribe native RNA templates into long representative DNA copies. The DNA synthesised has always been small compared with the viral RNA template and, furthermore, a small portion of the genome is represented in a greatly disproportionate amount. Last year some laboratories seemed to have come up with a solution to this problem: the reverse transcriptase under the then routinely applied enzyme assay conditions was starved of triphosphates (Collett and Faras, *J. Virol.*, **16**, 1220; 1975; Rothenberg and Baltimore, *J. Virol.*, **17**, 168; 1976). All one had to do, apparently, was to raise the triphosphate concentration, which resulted in a higher yield of long complementary DNA—admittedly, still in a very low absolute yield. I was probably not the only one who was told, when this news was brought home from a tumour virus meeting, that experiments under the new conditions were prohibited because of the expense of the isotopes! There seemed to be a way round when it was later published that a carefully optimised detergent concentration for disruption of virus would also result in a better

yield of long complementary DNA (Junghans, Duesberg, and Knight, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 4895; 1975). For a year radiochemical companies must have earned a fortune from deoxyribonucleoside triphosphates, as complementary DNA was being synthesised in the presence of high triphosphate concentration in many laboratories with the aim of obtaining restriction endonuclease maps of the viral DNA and of making more representative DNA probes for hybridisation experiments.

Now it becomes embarrassing: recently it turned out, that the problems were not caused by too low a triphosphate concentration but too high a magnesium concentration (Rothenberg and Baltimore, *Cold Spring Harbor Tumor Virus Meeting*, 1976). Magnesium chelates the triphosphates so that they cannot be used by the reverse transcriptase—a problem which can certainly be overcome by increasing the triphosphate concentration. If the observation turns out to be true, lowering the magnesium concentration will obviously do equally well. It may be a consolation for all those researchers who are tempted to consider themselves stupid for not having found this answer themselves that it had to be a laboratory like Baltimore's to come up

with it. However, the prevention of precipitation of triphosphates is not the only effect of magnesium. That "Mg is RNase"—a slogan known in many laboratories—may also be involved. Degradation of the template certainly presents an important problem in its transcription. This idea is supported by another recent study from Spiegelman's laboratory, which shows that addition of pyrophosphate—which probably inhibits RNase—to the reaction mixture gives a high yield of DNA complementary to poliovirus RNA used as a template (Kacian and Myers, *Proc. natn. Acad. Sci. U.S.A.*, **73**, 2191; 1976). This result will prompt all those who use reverse transcriptase as a tool for synthesising complementary DNA for hybridisation studies to apply these simple conditions immediately. The product obtained however, is single-stranded DNA; why no double-stranded DNA is synthesised remains unclear. This poses problems for subsequent analysis with restriction endonucleases which predominantly recognise double-stranded DNA.

Why was this work performed with poliovirus RNA? Analogous experiments with genomes from RNA tumour viruses are already in progress. Do they present greater difficulties than those described for poliovirus RNA—which is about the same size as a subunit from an RNA tumour virus (10,000 nucleotides)? The genome of RNA tumour viruses is known to contain obstacles for an enzyme which tries to read its way through completely. It runs into stop signs probably caused by the secondary structure of the RNA. This has recently been reported by Haseltine and coworkers from Baltimore's group who classified the DNA into discrete size groups (Haseltine, Kleid, Panet, Rothenberg and Baltimore, *J. Molec. Biol.*, **105**, 107; 1976). The DNA chains of discrete lengths are all initiated with the tRNA primer at a unique site along the 35S genome and grow by linear extension. The predominant size of the DNA is about 100 nucleotides. Surprisingly, even the longest DNA pieces obtained, (about 3,000 nucleotides) do not contain poly(A) transcripts from the 3' end of the genome. If one tries to compare these biochemical data with the beautiful electron microscopic pictures of the viral RNA which have become available from Davidson's group (Bender and Davidson, *Cell*, **7**, 595; 1976), no correlation between the lengths of the DNA size classes and the structure of the RNA can be detected. The complicated secondary structure of the RNA genome remains at present a specific problem for synthesis of a DNA provirus *in vitro*.

Another problem involved in

reverse transcription of the RNA tumour virus genome is caused by the unexpected position of the RNA primer. Why is the tRNA primer located close to the 5' end of the genome if the polymerase is supposed to start there for reverse transcription of the whole genome (Taylor and Illmensee, *J. Virol.*, **16**, 553; 1975)? There remains only a short way to go for the enzyme (from 3' to 5') until it hits the end of the template. The unexplained location of the primer immediately raised the suspicion that this was the reason for the small size and the repetitive nature of the DNA synthesised *in vitro*. It has recently been shown clearly that in fact the region between the primer and the 5' end of the genome was preferentially copied (Cashion, Joho, Planitz, Billeter and Weissmann, *Nature* **262**, 186; 1976). When fingerprinting the RNA protected by the DNA product they were able to identify the region of the RNA by means of the "cap" which is specific for the 5' end. A series of models have popped up immediately which postulate mechanisms by which the reverse transcriptase could still manage to make a complete DNA copy of the genome. A sequence complementary to the initially synthesised DNA piece may be present at the 3' end of the RNA genome which would allow circularisation of the RNA or bridging between two RNA subunits and continuation of the reverse transcription towards a complete copy. The first evidence for such a model was presented by Haseltine during the European Tumor Virus Meeting in October in Switzerland. His earlier observation mentioned above, that the polymerase jumps to the 5' end of the genome without copying the poly(A) region, gives indirect support to his newest result. A highly conserved constant region on the RNA genome next to the poly(A) tract at the 3' end which had been found in Weissmann's and Duesberg's laboratories while mapping the genome, could be a candidate for the complementary sequence. The chance of matching sticky ends in a test tube would then determine the yield of long DNA transcripts and may be low. This could be another reason why complete reverse transcription of viral RNA *in vitro* is difficult to achieve.

However, one should be able to circumvent this difficulty by applying a simple trick. Controlled heating of the viral RNA will knock off the tRNA primer and a new primer (oligo(dT)) could be bound to the poly(A) stretch at the 3' end of the genome. Why does the reverse transcriptase not synthesise a complete DNA provirus now? Other factors are possibly required? Unwind-

ing proteins involved in DNA synthesis have been well-known in bacteria for a long time and have recently been described in mammalian systems (Herrick and Alberts, *J. biol. Chem.*, **251**, 2124; 1976). Such proteins may be required for extending the RNA template or the complementary DNA for double-stranded DNA synthesis. Recently, the stimulating effect of some DNA binding proteins from chicken cells on the avian viral reverse transcriptase has been reported (Hung and Lee, *Nature*, **259**, 499; 1976). A protein factor associated with plasma membrane preparations from chick embryo cells also stimulates the avian reverse transcriptase (Padhy *et al.*, *Nature*, **262**, 802; 1976). A virus structural protein may even be a candidate for a factor involved in reverse transcription. □

Fire on heathland

from Peter D. Moore

A SECONDARY effect of the hot, dry summer in Britain has been the incidence of large numbers of fires, particularly on heathland and moorland areas. Some areas affected are of high biological interest and value, and conservationists must be asking themselves what the long term effects of these fires will be upon our flora and fauna, and also whether anything could have been done to prevent them.

Especially serious are those fires which have affected our National Nature Reserves, many of which are managed by the Nature Conservancy Council (NCC). These areas have been selected for their diverse biota and are usually the best remaining examples of particular habitats in Britain. Therefore damage to these areas is a matter of great concern. One of the worst examples of such damage during the summer occurred on the Hartland Moor National Nature Reserve in Dorset. This 637 acre reserve near Wareham is largely occupied by dry, *Calluna* dominated heathland. Several rare and diminishing species are found on the reserve, such as the Dorset heath (*Erica ciliaris*) and two reptiles, the sand lizard (*Lacerta agilis*) and the smooth snake (*Coronella austriaca*). As a result of the fire the original population of about 800 sand lizards has been reduced to an estimated 30. The destruction of the habitat has meant that the remaining animals have little chance of survival in the wild, so they have been captured and are being kept in vivaria at the University of Southampton until it is possible to reintroduce them. Even fewer smooth snakes have survived.

This fire occurred despite rigorous precautions. A ploughed, perimeter firebreak isolated the reserve from surrounding areas and there are internal, mown firebreaks. Unfortunately, a public right of way crosses the reserve and the fire began inside the perimeter. The internal breaks proved inadequate as a result of the drought.

Heathland is a habitat which is basically maintained by fire, so its recovery will undoubtedly proceed as in the case of past fires. There are three features, however, which make this fire particularly unfortunate. First, the extent of the burn; about 450 acres (over two thirds of the reserve) have been destroyed. This may slow down the processes of reinvasion and means that the refuge areas are small. Second, the involvement of reptiles which already have fragmented distributions. The heathlands of Dorset are now reduced to about nine extensive tracts and reinvasion from one area to another by lizards and snakes is unlikely because of the barriers of unsuitable land separating the heaths. A third unfortunate feature is the intensity of the fire, which has a serious effect upon the peat areas, such as the valley mires and flushes on the reserve.

Peatlands are particularly susceptible to fires during droughts, and several important British peatland sites have been damaged this summer. Parts of Thursley Common in Surrey are an example, also the raised bog of Glasson Moss in Cumbria. In a raised bog the growth of peat-forming plants and the subsequent deposition of their litter can raise a dome of peat four or five metres above their surroundings. The impedance of drainage by the peat mass results in a raised water table within the dome. Glasson Moss is a good example of such a mire. Part of it is managed by the NCC as a National Nature Reserve and this part (about 140 acres out of a total 600) has been very badly damaged by fire during the summer. Its vulnerability is obvious, being surrounded by old, partially drained peatlands and agricultural land.

Glasson Moss was surveyed for the NCC by Grieg (unpublished report to NCC: 1975) and, although he found that the central areas of the dome were of high quality botanically, he considered that this quality had deteriorated in recent years. It is believed, for example, that peripheral drainage is lowering water tables in the central area and it is known that the surface was badly burned in 1969. The whole *Sphagnum pulchrum* / *Rhynchospora alba* / *Drosera anglica* assemblage, typical of the central dome, is now becoming very scarce on Glasson Moss. Glasson Moss is not alone. Borth Bog in the Dyfi Estuary National Nature

Reserve, West Wales, has been burned again this year and this has become almost an annual event. Many such fires are known to be started deliberately by local farmers who often believe it to be a sensible land use practice in such areas, though often for purely superstitious reasons. At Borth Bog two plants are nearing, or may have reached extinction. These are the bog moss (*Sphagnum imbricatum*) and the brown-beaked sedge (*Rhynchospora fusca*); the latter was once widespread on the reserve but has not been seen there for the past ten years.

At both Glasson Moss and Borth Bog, the fire prevention measures are inadequate. At Glasson, firebreaks around the central area are mown, but this does not prevent the movement of intense fires during dry periods. At both sites there is a case for considering the construction of wide, water-filled ditches around the reserve boundaries. This would be expensive, but may be the only means of saving these vulnerable habitats.

There is also a need for more research into the recovery of peat surfaces from burning and its influence on species composition. Evidently such bogs have suffered some degree of burning in the past and have recovered. It is instructive to inspect the diagrams of exposed peat profiles from Danish raised bogs recently published by Aaby (*Nature*, 263, 281; 1976) which have charred layers running along certain horizons dating from Neolithic and Bronze Age times. The raised bogs recovered from these traumatic episodes, but what will be the outcome of recurrent, sometimes annual fires?

The Nature Conservancy Council in Britain has the responsibility for acquisition, management and maintenance of Nature Reserves and also for the commissioning of relevant research. Its record on the survey and on the acquisition side is quite good, but its success in the maintenance of acquired Reserves is not outstanding. True, conditions have been unusual this summer, but some of the damaging episodes, such as the burning of raised bogs, are regular, predictable events and the NCC must accept some responsibility for failing to take adequate preventative action in good time. No blame for this neglect can be placed with the regional and field staff who have done everything possible within their present limited economic and physical resources. One cannot help feeling, however, that in the case of mature, self-sustaining ecosystems like the raised bogs the NCC considers its responsibilities to end with acquisition and neglects the assignment of funds to their protection. Let us hope that the sad events of this summer will lead to

the redeployment of money and manpower from the administrative to the field management and research aspects of nature conservation. We need fewer paper clips and more gum boots. □

The blind white fishes of Persia

from a Correspondent

A NEW species of blind loach captured in the Zagros Mountains, Iran, has recently been described by Greenwood (*J. Zool., Lond.*, 180, 129–137; 1976). In itself this is both interesting and remarkable, but it also represents the successful culmination of a quarter-century of interest in Iranian eyeless fishes by their collector, Mr Anthony Smith.

In 1950 Mr Smith led a party of students from Oxford University on an expedition to Kirman in Iran; one of their objectives was to study the fauna of the qanats, artificial underground aqueducts which convey water from the foothills of the mountains to the plains villages. In particular, they hoped to capture fishes which literary sources suggested were blind and depigmented and lived in the channels. On the expedition's return, Smith published a most entertaining account of their adventures (*Blind White Fish in Persia*, Allen and Unwin, London; 1953). Unfortunately, and despite this title, the expedition failed to catch any blind fish (although apparently fishes were found in the qanats they were not described or identified).

In the interval between planning the expedition and its accomplishment, however, Bruun and Kaiser (*Dan.*

scient. Invest. Iran, 4, 1–8; 1950) published a description of *Iranocypris typhlops*, found in 1937 in a subterranean water system outlet in the Zagros Mountains in southwestern Iran.

In 1976 Anthony Smith returned to the area, and visited the well-like outlet in which *Iranocypris* had been collected. Here he caught three colourless fishes, two of which he brought back to Britain. One of them proved to be *Iranocypris typhlops*, the other, surprisingly, was a loach new to science which Greenwood has just named most appropriately in honour of its collector, *Noemacheilus smithi*.

The loaches are freshwater fishes of wide distribution in Eurasia (although they are also found in north-east Africa); they belong to the order Cypriniformes which also includes the carp-like fishes. Although numerous species are known, particularly in the Asiatic region, this is apparently the first typical cave-dwelling noemacheiline loach to be recognised. Earlier authors had discussed loaches in Assam and an unspecified part of India which had been found in caves, but in neither case were the specimens completely depigmented, nor were the eyes greatly reduced (although there seems to be uncertainty as to whether the latter population showed eye reduction at all).

Noemacheilus smithi, however, is as typical a cavernicolous fish as any described. Its eyes are completely absent (as far as macroscopic examination could establish); the orbit on dissection appeared to be a shallow depression filled with minute fat globules. In life, its coloration is dead white suffused with pale pink, the viscera are visible through the abdominal wall, and the redness of the gills can be discerned through the gill

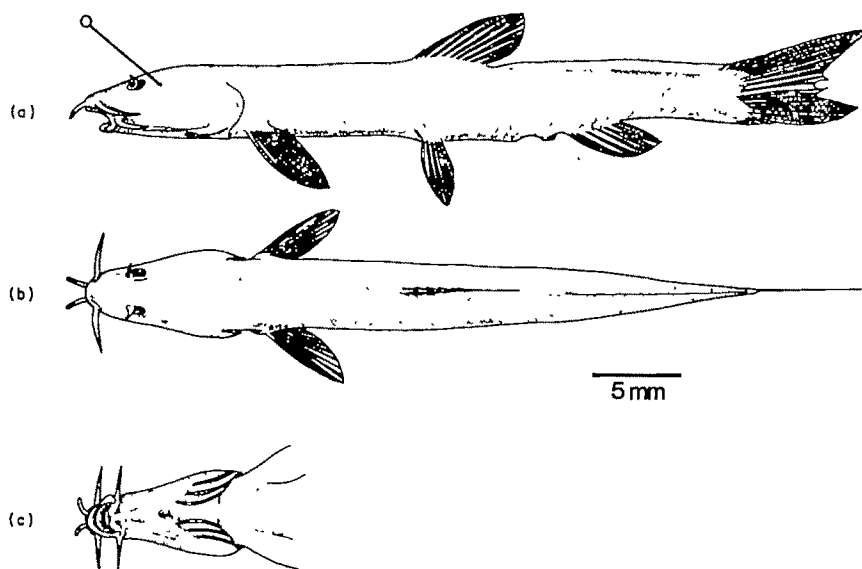


FIG. 1. *Noemacheilus smithi* sp. nov., holotype. (a) Lateral view (b) Dorsal view (c) Ventral view of head O -- position of orbit

cover. In other respects it is similar to other noemacheiline loaches, but the body is more slender than in most species, and there is a long, rather deep adipose ridge along the back behind the dorsal fin. The barbels around the mouth are, if anything, shorter and thicker than in other related loaches.

In a sense this discovery whets the appetite for more exploration in the area. The site in which both blind fishes were discovered is a well-like outlet, fully exposed to light, and in no way a cave. This poses the question, where are these fishes really living? Presumably the well is in contact with a subterranean water system in which both blind fishes live in some numbers. This, as yet, has not been discovered, but it could prove of great interest as this site where two fully-adapted cave fishes co-exist is only paralleled in the Mammoth Cave, Kentucky, a well explored system. Perhaps future exploration will yield further cavernicolous animals in Iran.

Iran's Department of the Environment, so helpful to Smith on his journey, has sent biologists to the area twice to collect further specimens. They found two more of the previously undescribed loach, which are currently being examined at Colorado State University, and plan a further expedition to the cave outlet this winter when water levels rise at the beginning of the rainy season. □

The theory and practice of star formation

from Ant. Whitworth

A Symposium on Star Formation was held by the International Astronomical Union at the University of Geneva on September 6-10.

STARS have formed in the past, and are continuing to form. Of this much we could assure the puzzled Huckleberry Finn who pondered "whether they [the stars] was made, or just happened": but of little more. Ultimately, self-gravity must drive the enormous contractions which convert diffuse gas into new stars. But the critical circumstances of this contraction—as the material destined for stardom overcomes the inhibitions posed by the internal thermal pressure, magnetic stresses, centrifugal acceleration, tidal disruption and turbulence—are extremely uncertain. The standard belief

is that most stars form in clusters, and the primary aims of star formation theory are then to predict, first, the distribution in mass, and in phase-space, of stars formed in a cluster, and second, the evolution in time of the external appearance of a star cluster during formation. To this end, four stages might usefully be distinguished: (1) the creation of a massive proto-cluster cloud, (2) the separation of individual protostars from such a proto-cluster cloud; (3) the contraction of a protostar to form a star, and (4) the interaction of a newly formed star with surrounding residual matter until the star becomes visible at a distance as a nonaccreting, unobscured, main-sequence star. (This fourth stage is included because it provides almost all the observable evidence of star formation).

At the symposium it was clear that, although the recent flood of observational data throughout the electromagnetic spectrum has tried many established star formation theories and spawned several new theories, much work lies ahead before star formation will be understood. For instance, the role of a spiral density wave and its associated galactic shock in initiating formation is very uncertain: whether it be to force a phase-change on the interstellar gas, thereby converting intercloud gas into clouds; or to assemble massive proto-cluster clouds by running together pre-existing small clouds; or simply to trigger by compression the gravitational collapse of pre-existing massive clouds. If we introduce the equally uncertain—and presumably coupled—role of magnetic flux, cosmic rays and their associated instabilities, we have already posed a long chain of unanswered questions and are still a long way from forming stars. B. Bok (Steward Observatory, University of Arizona) pointed out that there is extensive continuing star formation in the Magellanic Clouds; nowhere is the evidence more clearly laid out in the sky for the observer; yet there is no clear indication that star formation is very intimately involved with spiral structure, let alone an associated shock.

If such questions are put aside, and the existence of a gravitationally bound proto-cluster cloud is adopted as a starting point, we confront the problems of separating out individual protostars: by fragmentation or condensation, accretion or accumulation. Are protostars formed by an hierarchical fragmentation sequence which breaks the proto-cluster cloud up, in a cascade towards increasingly small, dense pieces, as originally envisaged by Hoyle? More probably, contemporary protostars condense directly out of a relatively low background density

($n \lesssim 10^4 \text{ cm}^{-3}$) due to gravothermal instability. Certainly no clear evidence for hierarchical fragmentation has been found in radio line (for example, CO) mapping of molecular clouds, in spite of the use of ample resolution—although any information would surely be a complicated convolution of density, temperature and optical depth structure. The only clear observations of fragmentation during star formation are (1) the high incidence of binary and multiple star systems, which implies that the inception of a typical protostar occurs in circumstances dominated by angular momentum, and (2) young clusters compounded by OB associations.

C. Lada (Center for Astrophysics, Harvard University) and B. Elmegreen (Princeton University Observatory) drew attention to a significant pattern observed in star formation regions. Ideally, this pattern is a regularly spaced alignment, parallel to the galactic plane, of first an elongated, quiescent molecular cloud; then an excited molecular cloud exhibiting signs of internal activity and star formation (infrared sources, masers and compact radio HII regions); next a compact OB association collectively exciting an extended optical HII region; following this an expanded OB association; then an even more expanded OB association. They submit that this sequence may arise as follows. An OB association formed in the front end of the cloud excavates an HII region, and sends an ionisation front back into the cloud. This ionisation front is preceded by a shock front, and after a time there is a shocked layer of neutral gas between these two fronts, which is sufficiently dense and cold for a new OB association to form by gravitational instability. And so the process repeats itself with a period of $\sim 2.5 \times 10^6 \text{ yr}$, producing a string of OB associations at intervals of $\sim 15 \text{ pc}$.

Given the considerable uncertainty which surrounds the inception of protostars, it is very difficult to predict the statistics of newly formed stars. C. Low and D. Lynden-Bell (Institute of Astronomy, Cambridge) have made a detailed evaluation of opacity-limited limit of $\sim 0.007 M_{\odot}$ for the local con-fragmentation and derive a lower mass temporary gas. J. Silk (University of California, Berkeley) has reproduced the Salpeter initial mass function (IMF) on the basis of opacity-limited fragmentation, and has also made several other quite definite predictions which occur with observational inferences: small stars form first at the centre of a proto-cluster cloud and heat up the outer layers, thereby increasing the Jeans' mass; consequently massive stars form later near the cloud surface.

Formation of the most massive stars,

however, is commonly assumed to involve an appreciable accretion phase. The upper mass limit is then attributed to an accretion cutoff, when the underlying star develops a sufficient ratio of luminosity to mass, that radiation pressure (acting through grains) halts the inflow. S. Strom (Kitt Peak National Observatory) suggested that accretion may instead be halted by a strong stellar wind. But the essential point is that Silk's IMF for fragments may not be completely relevant if the mass of the final star is much less than that of the fragment, the balance being contained in an unaccretible cocoon, as envisaged above (or perhaps in a disk which serves as a sink for the star's unwanted angular momentum). The final mass may then depend more strongly on the critical accretion parameters (density, opacity and velocity dispersion in the ambient gas).

Moreover, simple arguments show that if, at its inception, the protocluster cloud contains realistic measures of frozen-in magnetic flux and minutely conserved angular momentum, then subsequent contraction and fragmentation will amplify magnetic and centrifugal stresses until they assume dominant roles in the dynamic evolution. I. Mestel (Astronomy Centre, Sussex) pointed out that until—or unless—the charged particle density be-

comes very low, the gas is coupled to the magnetic field, and so clouds must tend to contract along the local field-lines; if the gas remains coupled to an attached, large-scale field for long enough, magnetic stresses may transport angular momentum from a cloud to its background, thereby postponing the development of centrifugal stresses until individual stars approach the main-sequence; by this stage the gas is recoupled to the field, and magnetic braking may again act to slow the rotation of the newborn star.

In conclusion, existing theory can say nothing about the overall efficacy of star formation. So many possible contributing processes can, at best, be evaluated in terms of their energetic feasibility, and seldom in terms of their competitiveness. Observations indicate that star formation in the solar vicinity presently converts ~5% of the interstellar medium into stars at each passage through the spiral pattern. Various attempts have been made to fit the mean rate of conversion of interstellar matter into stars with a law of the form $d\ln(\rho)/dt \propto \rho^n$, where ρ is the mean space density of interstellar matter, and n is to be determined by fitting observations. The results are conflicting and probably no such simple relationship exists where star formation is concerned. \square

ozone chemistry is not a theoretical myth but a reality in the stratosphere. It is not clear, however, how much of this observed chlorine comes out of the CFMs 11 and 12 and how much from natural sources like CH_2Cl , CCl_4 , and so on; what are the other competing reactions in which Cl atoms get involved to make them inactive for ozone reduction; and lastly, how many of them are removed from the stratosphere before they meet the ozone molecules. These are the uncertainties which led the NAS panel to put a factor of 10 on the uncertainty in their estimate of ~7% for the eventual reduction of ozone by CFMs. This implied that the eventual reduction of ozone could be anywhere between 2 and 20%. Several of these uncertainties were discussed at the conference. It appeared, for example, that the removal of Cl by the production of and eventual precipitation of the ClONO_2 molecule may not, after all, be such an efficient mechanism. Other uncertainties, however, remain. One hardly expected them to be answered only two days after the release of the NAS report. But one thing became very clear; with the amount of scientific activity in this field, this time next year the uncertainties will certainly be reduced by a significant amount.

The other scientific issue discussed at the conference was that of N_2O ; its sources and sinks near the ground and its eventual transport to the stratosphere. The problem, if it exists, could be greater than that of CFMs. It appears that N_2O , a product of biological denitrification in the soil, does not get easily "rained out" in the troposphere, diffuses on to the stratosphere, reacts with atomic oxygen, gets converted to NO and NO_2 , which then acts as a catalytic agent to destroy ozone in the upper atmosphere. Does this really happen or not, is of course an interesting scientific question for the students of ozone chemistry in the stratosphere. But the problem becomes greater when it is pointed out, as was done by Michael McElroy of Harvard last year, that the ever-increasing use of fertilisers may considerably accelerate the release of N_2O to the atmosphere with an eventual impact on ozone much greater than that predicted for supersonic aircraft, or the CFM.

Is this a real problem? We do not yet know. First, one needs much more precise information on the sources, sinks and life time of N_2O in the "natural atmosphere". What are the fluxes over the land and over the oceans? How much is the air-sea exchange? What is the life time of N_2O in the troposphere? What is the fixation time on the surface? Again, as in the case of CFMs, the theory seems

Stratospheric decision making

from S. I. Rasool

An International Conference on the Stratosphere and Related Problems was held at Logan, Utah on September 15-17, 1976.

It is refreshing to come out of a two and a half day international conference with a certain sense of accomplishment. This was partly due to the theme of the conference we not only discussed the physics and chemistry of the stratosphere, but one day was dedicated to questions related to the dynamics of decision making on the stratosphere. Nowadays when the stratospheric scientist has suddenly become an important individual, to both the media and the policy makers, interaction between the three was productive and satisfying, helped along by well-chosen speakers and panel discussions in which the major issues raised at each session, were if not solved, at least clarified.

In particular the conference took place two days after the much awaited release of the National Academy of

Sciences (NAS) report on the CFM problem. (*Nature*, 263, 268; 263, 723; 1976). After this conference and after the report where do we stand on the question of stratospheric impact of man-made products and what should be our attitude towards "regulation" of these products?

As for chlorofluoromethane (CFM) release, much progress has been made since the publication of the now famous paper by Rowland and Molina (*Nature*, 249, 810; 1974). We now know that most of the CFM 11 and CFM 12 ever released has accumulated in the atmosphere. We also know that a few per cent of them are reaching the stratosphere. There is also evidence that they are being dissociated, producing chlorine atoms which have a potential of catalytically reducing O_3 to O_2 . A major new set of measurements reported by J. Anderson (University of Michigan) confirmed, for the first time, the existence of Cl and ClO in the stratosphere. This was a crucial measurement that actually demonstrated that the chlorine-

to be in hand but the crucial measurements are just beginning to be made. As the Chairman of the Session, H. Schiff, put it. "We don't yet know whether the laughing gas problem is a 'laughing matter' or not".

The most exciting session of the conference was of course the one dealing with the dynamics of decision making and the topic under discussion was whether to "regulate" or "not to regulate" the use of CFMs 11 and 12 although the scientific evidence of the magnitude of their impact on ozone is still largely inconclusive. Policy makers from Washington, including the Chairman of President Ford's Council of Environmental Quality, discussed how decision making on issues of public policy proceeds in the face of scientific doubt.

About two million man-made chemicals now exist, and about 25 thousand are added each year. Since it is impossible to deal with them on a case-by-case basis, some general policies and philosophies are obviously needed. Those chemicals which have direct and immediate effects such as food poisoning or acute radiation exposure are of course easy to handle. For those chemicals whose effects are not immediately obvious and may occur several years or decades later, such as cancer-inducing products, or the CFMs, decisions on control have to be taken from a different perspective. In these cases public concern is not always an accurate indicator of acceptable risk and one has to keep in mind that often the values of the lives of the yet unborn are not assumed to be the same as those of our own. Sometimes it may also be unwise to postpone the decision until the effects can really be measured because a major impact may already have been made.

How then should we proceed? It was abundantly clear in the minds of the policy makers that although there remain valid doubts about the effects of CFM on the ozone layer, from the standpoint of public policy there remains no valid reason to postpone the start of regulatory procedures on the use of CFMs 11 and 12. They strongly suggested an immediate voluntary phase-out of non-essential uses of CFMs. This opinion was in direct conflict with that in the NAS report calling for a wait of up to 2 years depending on when the uncertainty can be reduced. The Academy panel was severely criticised for going beyond its charter and pronouncing judgement on a public issue. "There are no individuals better qualified to make scientific judgements than scientists. But scientists are no more qualified men than anyone else in making social value judgements," said Russell Peter-

son. "Moreover, this judgement carries with it added credibility simply because NAS made it and sets a strong public mood," added David Pittle, Commissioner of the US Consumer Product Safety Commission.

The debate between the scientists and the Washington bureaucrats was certainly educational for both. The scientists were reminded of their responsibility to present complex issues so that they could be understood by the public and that the uncertainty in their predictions is more evident than obscure. They were urged to place their own work (and bias) in perspective so that the media and public get a balanced view of the issues and answers.

For the scientists, so used to peer review and refereed journals, it was perhaps a little difficult to understand how one really assesses the importance of public opinion which is at once so volatile and so dependent on the media coverage of the news. We were assured by the Environmental Protection Agency that there were cost benefit analyses before any economic judgement was made and we were told that several months of public hearings take place before a judgement on the public opinion is made.

This conference was a satisfying affair. I think we all came home a little more reassured about each other and about each other's role in the eventual solution of the problem. □

Earth's core in Oregon?

from Peter J. Smith

LAST year Bird and Weathers (*Earth planet. Sci. Lett.*, **28**, 51; 1975) made the startling suggestion that josephinite, a rare rock consisting largely of nickel-iron alloy, may have originated in the Earth's core, or at least in the vicinity of the core-mantle interface. If this view were to be substantiated it would be of more than passing interest, for proven examples of core material in the crust are not exactly common. It would also carry with it certain implications about the Earth's ability to transport material over great vertical distances. Indeed, Bird and Weathers proposed specifically that josephinite may have been raised from the core-mantle interface in a mantle plume, carried into the lithospheric mantle by diatremes and emplaced into the upper crust by ophiolite obduction. But none of this exoticism was destined to go unchallenged; and Dick and Gillette (*Earth planet. Sci. Lett.*, **31**, 308; 1976) have now taken Bird and Weathers to task on a number of points which taken

together suggest a much less spectacular origin for josephinite.

The basis of the case put by Bird and Weathers was that the josephinite found as pebbles at Josephine Creek, Oregon, is distinct from the more common, albeit superficially similar, mineral awaruite. The chief differences claimed are that josephinite is much coarser-grained than awaruite and that, unlike awaruite, it contains two metal phases (nickel-iron alloy and α -Fe) and andradite garnet. Moreover, some josephinite samples also contain elemental silicon and a natural variety of the artificial substance $\text{CaO} \cdot 2\text{FeO}$, neither of which has previously been found in terrestrial rocks. Such differences were held to support the view that josephinite and awaruite have a different origin.

But even some of the facts are in dispute. Apropos of the reported discovery by Bird and Weathers of two metal phases, for example, Dick and Gillette claim that the metal in josephinite is all nickel-iron alloy, albeit with a "large bimodal compositional variation" between samples. There are indeed two metal phases, but one is awaruite and the other is taenite (rather than α -Fe), both of which are nickel-iron alloys. Dick and Gillette do admit that the association of the two metal phases with andradite in the samples analysed by Bird and Weathers is uncommon, although they point out that many of the pebbles contain only awaruite. They also quote a forthcoming report from Botto and Morrison which claims that there is in fact a complete gradation between awaruite and awaruite-taenite-andradite.

Next, Dick and Gillette throw doubt on the presence of elemental silicon in some samples which can be interpreted in other ways. In any case, they question the view of Bird and Weathers that the presence of silicon would preclude origin in the lithosphere, citing the production of impurity silicon in the smelting of iron as evidence of the element's ability to form stably at low pressures. The suggestion that andradite garnet/nickel-iron is not a stable assemblage (implying that the two components could not have formed together) is also disputed, largely on the grounds that Gustavson's (*J. Petrol.*, **15**, 455; 1974) experiments on the stability of andradite have been interpreted by some of his successors too uncritically and incompletely. And finally, Dick and Gillette suggest that the presence of diamonds in Josephine Creek, even if correctly reported (it is in fact in dispute), does not necessarily support the case that Bird and Weathers were making, if only because diamonds are representative of upper, rather than lower, mantle pressures.

Also relevant to that case are the dif-

ferences, or supposed differences, between the nature of the pebbles (which are placer deposits) and that of similar *in situ* material within the mass of the nearby Josephine Peridotite. The Josephine Peridotite, partly unaltered harzburgite and partly serpentinite, is apparently a segment of ophiolite emplaced by obduction. All parties agree that it contains awaruite in parts and that awaruite is formed by the serpentinisation of ultramafic rocks. They likewise agree that the Josephine Peridotite is the source of the pebbles.

But the consensus then breaks down. Dick and Gillette claim that both the *in situ* material and the pebbles are awaruite and (bearing in mind the nearness of the pebbles to a zone of intense shearing, serpentinisation and igneous intrusion within the Josephine Peridotite) that both are the products of low-temperature hydrothermal metamorphism and serpentinisation of the peridotite. Such differences as there are between the two (for example, it is acknowledged that the pebbles have unusually large grain size) represent varying conditions imposed by igneous

intrusion during serpentinisation.

Bird and Weathers, on the other hand, claim that the differences between the pebbles and *in situ* material are serious enough to imply a different origin (and to require separate names) for the two. Having acknowledged that the source of the pebbles is the Josephine Peridotite, having agreed that the *in situ* awaruite is the result of serpentinisation and having proposed a core origin for the pebbles, this would appear to put Bird and Weathers in the position of saying that the Josephine Peridotite once contained (and perhaps still contains) two distinct but closely related nickel-iron alloys whose zones of origin were separated by more than 2,000 km vertical distance.

Obviously it is possible. For example, a mantle plume could have been feeding the accreting plate margin from which the Josephine Peridotite is derived. But is it credible? If so, the exciting possibility of core material at the Earth's surface remains. If not, the balance of the argument must be in favour of Dick and Gillette who in any case have Occam's Razor on their side.

details of a number of sophisticated applications in nuclear physics. Following on from this, M. Lopenen (Otaniemi) introduced a lively debate on the problems and pitfalls of secondary thermometry.

One of the most fascinating, and perhaps also the most important, topics of the conference was reserved for the final day when K. W. Taconis (University of Leiden) and F. A. Staas (Philips, Eindhoven) talked about the new cycle in which instead of extracting ^3He from the dilute phase in the mixing chamber using a still, ^3He is pumped in through a superleak. This technique offers the great advantage that the dilute and concentrated phases counterflow in the same tube, rather than in separated tubes, so that no heat exchangers are needed. The simple Leiden machine, which reached 8 mK very soon after having been commissioned, was discussed in detail. Hybrid machines in which a "Leiden" mixing chamber is piggy-backed on a conventional refrigerator, and both ^3He and ^4He are separately circulated, as developed at Philips and Grenoble, were also considered. The meeting ended with an all-too-short session presided over by E. J. Varoquaux (Orsay) on the actual practical problems associated with operating refrigerators.

Notwithstanding the astonishing progress of the past 10 years, the dilution refrigerator is clearly still capable of significant improvement. If, as one suspects, commercial machines capable of reaching 5-6 mK soon appear on the market, it will be very largely a result of the beautiful research and development work currently in progress on this side of the Atlantic. □

Millikelvin technology

from P. V. E. McClintock

A Europhysics Study Conference on Dilution Refrigeration and its Applications was held at Lancaster University, UK, on September 25-27 1976. The meeting was organised by D. Thoulouze (Grenoble) and G. R. Pickett (Lancaster).

It is now more than a decade since the first dilution refrigerators were built. During this period the machines have become the standard means of reaching temperatures between 10 and 300 mK in a continuously operating mode and, moreover, the technique has spread far beyond the area of purely low temperature physics and is being widely used in a number of other fields.

Dilution refrigerators operate on a mixture of liquid ^3He and ^4He making use of the property that, at low temperatures, the liquid separates into two phases, usually known as the concentrated phase and the dilute phase. The concentrated phase consisting of almost pure ^3He floats on top of the dilute phase, which is composed of a gas-like assembly of ^3He atoms moving freely in a "background" of superfluid ^4He . Thus the movement of ^3He atoms from the concentrated to the dilute phase is very much like the evaporation of an ordinary liquid, and is

accompanied by a similar cooling effect.

The conference started with an introductory session led by H. E. Hall on what has become accepted as the conventional cycle: the "General Motors, rear-wheel-drive refrigerator", as he called it. In this cycle, ^3He is drawn out of the dilute phase by a heated still and the cooling effect arises from a balancing flow from the concentrated phase. The process is sufficiently well understood that machines capable of reaching 10-12 mK are available on the commercial market. The two sessions which followed led by G. Frossati (Grenoble) and A. Th. A. M. de Waele (Eindhoven), discussing the optimisation of this technique, provided what in many ways formed the high spot of the conference. It was particularly impressive to hear that the machines developed in Grenoble are able to reach 4 mK (not very far above the liquid ^3He superfluid transition) as a matter of routine and, furthermore, that their performance is in reasonable agreement with calculation.

On the second day some of the recent practical applications of dilution refrigerations were described in sessions led by R. E. Packard (University of California, Berkeley) and T. O. Niinikoski (CERN), and included



A hundred years ago

RUSSIAN newspapers announce the death of M. Chekanoffsky, who, exiled in Siberia, has spent more than ten years in the geological exploration of the country, and recently returned from his travels on the Olenek and the shores of the Polar Sea, to St. Petersburg, where he was engaged at the Academy in the description of his immense collections. He was found on October 10 dead in his room, and it is supposed that he poisoned himself. From *Nature*, 15, November 9, 50; 1876.

WHATEVER happens now, we cannot blame the Marxists for demystifying science. It's true that they were first in the field in England, shocking the bourgeois at the British Association at Durham in 1970 with their slogan: "Science is not neutral". But the point was soon reiterated to greater effect, not merely by Lord Rothschild's gauntlet, but even more by Sir Frederick Dainton's intended smooth covering. It was he who mentioned a "dangerous and corrupting 'ivory towerism'", to be countered by a continual awareness of "national needs and objectives" by those planning basic and strategic science. And since then we have all learned that the language of science is priorities, with a grammar of shrinking budgets.

Of course, the points are made with quite different motives from the two sides our established leaders want more 'civic responsiveness' from science, while the counter-slogan is 'social responsibility'. And the analyses of the situation of science diverge quite radically. Those who would manage science act on behalf of a social order assumed to be basically good, and capable of further improvement by the proper application of science. The Left critics exhibit science as a microcosm of an unjust society, and expose its hypocritical pretensions to superiority.

Indeed, the general impression that may be conveyed by the new double-decker edited by the Roses, *Ideology of/in the Natural Sciences*, is that of a many-sided assault on cherished illusions. For them and their collaborators science is neither innocent, ennobling, or even nice. The effect on a thoughtful scientist could well be similar to that on a benevolent, complacent male who picks up the *Spare Rib* that his women-folk avidly read, and discovers what he looks like from below. Although he may consider himself eminently fair in playing the game of social life, he is there told that the rules are rigged, for him and against the others. And if he is smitten with concern and guilt, he also learns that there is no easy cure for maleness as a social disease.

Similarly, those who enjoy the benefits of participation in the upper reaches of the institution of science are typically white and middle-class as well as male; thus they belong to a group whose security, until now enormous, is now decreasing markedly. Moreover (and this is an important thrust of the book), the character of the class of scientists is closely bound up with that of the science that they produce or apply. In its objects, methods and functions, our science is a creature of its matrix in a particular sort of class society. Stripping away the ever thinner veneer of 'pure science', these critics find that science is supported for its

Book review supplement



contribution to two ends: production, industrial and military, through physical inventions and discoveries; and social control, through its results, methods and above all ideology.

In this last point the authors initiate a major advance in Marxist thought.

Assault on cherished illusions

J. R. Ravetz

Ideology of/in the Natural Sciences (Two volumes.) Edited by Hilary Rose and Steven Rose. (Macmillan London, November 1976.) *The Political Economy of Science* Pp. 320. *The Radicalisation of Science*. Pp. 260. Each volume hardcover £10; paperback £3.95.

Until now, the Marxist tradition (along with positivism and rationalism) has assumed natural science (as best exemplified in physics) to be above the contradictions of society and independent of false ideology; from that firm basis, intending revolutionaries could proceed with certainty to transform the social world. The authors here are determined to exhibit the socially conditioned ideology 'of/in' natural science, without collapsing into relativism or irrationalism. This is a task of some delicacy, here only begun.

But a beginning there must be, and the authors have provided it, mainly (and properly) by accounts of areas they know from their own experience. These books have advantages and drawbacks of a largely amateur collection; there is repetition between essays

(as well as divergence of approach and doctrine), some looseness of organisation within them, and occasional bursts of rhetoric not intended for most of the readers of this present review. But generally, they are refreshingly free of jargon and bombast. And there are many insights to be gleaned in them, I shall try to convey some of these.

To accomplish this thoroughgoing critique, there must be a very broad definition of 'science', really encompassing all of theoretically trained problem-solving expertise. The most visible part, academic 'pure' natural science, is in this analysis reduced to a small, self-deluded sector. In one of their more breathtaking asides, the Roses remark: "By the end of the war, the autonomy of science had become a myth helping to ensure that those students with first-class degrees remained in the universities and those with seconds went into industry" (*Radicalisation*, p7.) Cruel, to be sure, but is it a complete caricature? And since there is no consensus on the boundaries of 'science', one must judge any proposed definition by the effectiveness of its use.

The Roses and their co-workers are not just demystifying and muck-raking. They are proclaimed Socialists, taking their inspiration from Marxism (although in what they call its "problematic inheritance"). Their essays are then both a criticism of capitalist natural science, and a demonstration of Marxist social analysis, as enriched by themselves. Their Marxism is the opposite of dogmatic; indeed it is easier to say precisely what it is *not* in terms

* Illustration of the "Book Wheel" taken from *The Various and Ingenious Machines of Agostino Ramelli*. Translated with an introduction by Martha Teach Gnudi and Eugene S. Ferguson. Pp. 604. (Scolar: London; John Hopkins University: New York, July 1976.) £50.

of the worries it relegates (environmental problems, and community and personal development), than what it is, by the articles of belief it maintains. Even the placing of the authors with respect to movements and powers in the contemporary world is rather vague, the book is dedicated to the "Heroic Peoples of Indochina", and there are occasional exhortations to workers; but otherwise it is all somewhat abstract. Even their citations to professedly Marxist literature on their subjects are very sparse. This independent policy has its benefits, of course; the authors are then entirely free to criticise capitalist culture on the basis of its own best values of liberty, decency and honesty—rather like Marx himself. Also, it enables them to move implicitly in some very interesting directions, of which more later.

What, then, can scientists see of themselves through this radicals' mirror? The Roses' leading essay "The Incorporation of Science" gives a synoptic view of the field (as their introduction gives admirable summaries of all the papers). The new conception of science, first described simply as 'big' but now as 'incorporated' or 'industrialised' subverts all the ideals of nineteenth century 'pure' science, and also many of those of the 'improving' tradition from Bacon to Bernal. But most academic studies of science (history, philosophy, sociology) are as yet only dimly aware of this transformation "from scientific community to scientific factory". Perhaps this is because they relate, not to the mass of *scientific workers* who are found to be indifferent to the hallowed 'norms' of science, but to the visible elite. This group can be identified; some 0.01% of the American scientific work-force of 2 million are the real decision-makers. And it is in their interest to maintain the myths of autonomy and 'public knowledge' as the ruling ideals of science. With this demystification accomplished, the book is launched on *The Political Economy of Science*.

An analysis of modern science-based industry that is broadly in line with classical Marxism is provided by Mike Cooley. He describes the proletarianisation of white-collar and technical labour, along with the continued dehumanisation of production-line work through Taylorism. His own experience in AUEW-TASS provides thought-provoking examples: computer-aided design, distorting the balance between the quantitative and the qualitative and leading to a stifling machine-enforced bureaucracy in the design process; attempts to restrict factory recruitment to men under 30 (they 'burn them up' in 10 years); the series of three strikes necessary to get heating and ventila-

tion for workers comparable to that provided for computers. This ultra-high technology industry aggravates many of the contradictions of capitalism analysed by Marx and also has created a few of its own: the rapid obsolescence of knowledge, and the extreme vulnerability of such systems to 'guerilla'-type action—be they works to rule at home, or Sino-Vietnamese warfare abroad.

The attempt to explicate science (as distinct from science-based industry) in orthodox Marxist terms is more demanding, a group of Italian physicists make a brave try, but it is not very illuminating for those not sharing the paradigm. The second half of their essay, based more on their personal experience, is more rewarding. By contrast, the less theoretically-orientated analysis of André Gorz provides a comprehensive radical sociology of scientific knowledge. He sees the system of scientific research from the outside, and accordingly can make criticisms that would be difficult for an established research scientist, however rebellious, to express.

For Gorz, all the features of science that render it the preserve of an upper-class minority, are solely to be explained by their social functions. Science itself is effectively defined by formal teaching and diplomas; traditional or self-taught craft skills have no standing against certified expertise. And only those "socially qualified to exercise authority" will succeed in the training system; thereby ensuring that this knowledge is not utilised by the wrong power. But in any event, most of the knowledge of science is alienated and irrelevant to life, in a unique degree. The people are totally excluded, and the experts are fragmented and helpless (and in the case of academic researchers, useless as well). It is then easy for science to be directed to answer some questions and ignore others (Gorz here mentions high technology medicine squeezing out old traditions and communal self-help). Merely changing ownership relations seems to make little difference, so long as knowledge (and the associated power) is hierarchical. Indeed, in passages that recall Rousseau and the Jacobins rather more than Marxism, Gorz rejects *all* science that cannot be shared among 'the people' as their own.

The discussion of 'ideological' conditioning of science starts with fields studied over past years by the Roses: IQ and 'biologism'. Perhaps because of one's prior familiarity, the feeling of "yes, but" increasingly obtrudes on one's reading of the well-argued essays. For example, we are told explicitly that theories linking race and IQ "provide an apparent scientific rationale for the existing social order", almost as if our rulers wanted or called for it. But the directors of our educational system

(surely an area of ideological formation and social control) have quite recently jettisoned the IQ as a means of selection, and this is nowhere mentioned. It is generally much more difficult for a radical critic to explain the virtues of a system than its vices; but to ignore the problem is not productive, nor even consistent with the authors' own standards. I must also remark that in the essay on 'biologism', with its catalogue of horrors of behaviour control from the USA, there is no discussion of the current Soviet conception of schizophrenia as a simple mental disease with obvious behavioural symptoms, including, of course, political deviance.

The really 'soft' natural sciences, such as reproduction technology and 'political ecology', are easy targets for analysis, but the studies here are not trivial demystifications. The real contradictions will not be removed by a reduction to 'class' terms; indeed, traditionalist Marxists fare as badly for their ecological blindness, as do the technical fixers of contraception, for their male chauvinism.

This Marxist approach, however, can really prove its power only in a case where the ideology is *not* apparent, where 'objectivity' seems to reign. Such is the case in physics; and J. M. Levy-Leblond delivers a broadside on that

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noble discipline. Of course, one finds factory research there (as at CERN), the sometimes vicious competition for degraded honours as the Nobel prize and the strange cult whereby scientific eminence is a token of practical wisdom. More than this, the author gets into the subject itself, noting its glorification of brainwork over handwork, the pure over the practical, the 'hard' and abstract over the 'soft' and particular. When this science is taken as the paradigm (as for most of this century), its reinforcing of social and sexual elitism, in the educational system and generally, is very strong. Wisely, the author shies away from discussing the relations of this ideology to the 'truth content' of physics; but for a demonstration of how deeply ideology can penetrate, this essay is a very good start.

When we come to *The Radicalisation of Science* the contradictions in the authors' program appear clearly. The Roses have a delightfully self-critical history of British Society for Social Responsibility in Science and its associated movements; but they would not pretend that this movement ever achieved any numerical strength, nor that it is likely to do so in the foreseeable future. In the most erudite essay in the collection, the American radical geneticists, Lewontin and Levins, grasp

the nettle of the Lysenko episode, which did so much damage to the Marxist movement among scientists after the war. They display the many factors that made Lysenko plausible at first: not merely the crisis in food supply, the institutionalised paranoia about 'wreckers', and the resentment against elite foreign-orientated Mendelian geneticists; but also the harshness and irregularity of the Russian climate, that made 'averages' less meaningful than in milder environments and so required different methods and criteria of adequacy in statistical work. For these authors the essence of the matter was that Lysenkoism was an abortive Cultural Revolution: it went wrong because of the particular causes (and also effects) of Stalinism. This might be interpreted as a cautionary tale for hyper-Left reformers of science; and comparison with earlier populist attempts, as the 'Jacobin science' in the French Revolution and the Paracelsians of the Puritan Revolution, would repay study.

How, then, could the true radicalisation of science be achieved? It is a pity that we are not given a successful case study as from Indo-China or Cuba, where a mainly political approach will have been tried. For otherwise, we are left mainly with the women and the inimitable Joseph Needham. (The moving essay by the American Black scientist tells us more about his problem than about an effective solution.) And quite suddenly, Needham does not seem so eccentric as previously in the company of professing Marxists. Of course, he is still developing philosophically, as young in spirit as ever. Here he takes on a new challenger, the counter-cultural Roszak; and like the great Middle Kingdom itself, absorbs and assimilates him. He does so by moving his own Taoism a bit closer to its mystical, revolutionary tradition.

Given Needham's insights, it is really quite simple: 'our' science (be it capitalist or European), is all *yang* and no *yin*; like our civilisation in general, its maleness is dangerously predominant over its femininity. The women's critique of science fits in perfectly here; one (Couture-Cherki) shows the extent

of discrimination and explicit piggery in French physical science, the other (Stéhélin) hints tantalisingly at a feminine cosmology that would enable women to escape the trap of becoming pseudo-males in order to succeed at male-shaped natural sciences. And if this seems fanciful, we can refer back to the analysis of physics by Levy-Leblond.

Of course, no-one has a recipe for *this* sort of radicalisation of science, at least no-one in the Marxist tradition. But is it really a more forlorn hope than transforming science and society by means of an industrial proletariat? After all, Marx *did* believe that ownership relations were the main fetters on the essentially beneficent forces of production; we now cope both with reactionary 'non-ownership' societies and with socially and environmentally malignant commodity production.

This just about brings us back to the worried male (perhaps a scientist!) who has discovered *Spare Rib*. It also may convey a lesson about social change, which is indicated in several essays here. Regardless of how clear or apparently 'objective' may be one's vision of the social world, one cannot simply ignore one's individuality and, from outside, engineer a solution to its problems. For each of us is part of the problem; and in resolving it we must discover (and perhaps demystify) ourselves. Perhaps the next step forward in the study of ideology '*off/in*' science will come from the full working out of this principle in connection with the accepted public knowledge of the natural world. In the meantime, these essays make a very good start; and the editors and authors may well be among the first to share in the next move of the game.

But when all this is said, where does such a neo-Marxist critique leave science? In getting from today to tomorrow, science has enough to do nowadays just surviving. It always happens that the more radical the analysis, the more irrelevant it is to ordinary experience, and the more useless it is as a guide to everyday practice. But change goes on everywhere frequently accelerating rapidly. If we are to understand the emerging radical insights about the social activity of sciences, in their content and contradictions, we must pay serious and sympathetic attention to those who are first trying to articulate them. For this instruction, even those who disagree deeply with the Roses and their colleagues will be indebted to them. □



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Methodologies and myths

R. V. Jones

Method and Appraisal in the Physical Sciences: The Critical Background to Modern Science, 1800–1905. Edited by Colin Howson. Pp vii+344 (Cambridge University: Cambridge and London, September 1976) £10 50.

"THIS volume constitutes the first collected edition of work so far done in illustrating an important new development in the philosophy of science, 'the methodology of research programmes'". So claims the editor of this collection of seven monographs which exemplify and examine the ideas of the late Imre Lakatos (who is the posthumous author of the opening paper) regarding the philosophy of science. Five of the papers deal with selected case histories, three in physics (kinetic theory/thermodynamics, wave theory of light, and relativity) and two in chemistry (oxygen/phlogiston, and Avogadro's hypothesis).

The self-styled 'importance' of the new development may make the reader bridle and he may well ask what a research "program" is. It suggests a deliberate plan by an individual or a group, and it ought therefore to exclude those developments in science where there has been no conscious planning; any attempts by subsequent

philosophers to fit such events into a plan will be falsely based. With these prejudicial observations I have attempted, as an experimenter interested in the history of science, to read the book.

As for the philosophy, there is so much unfamiliar jargon that I can only grasp at clues to determine whether or not it is worth mastering. Lakatos discerns four "logics of discovery", of which one is "methodological falsificationism", and his exposition is strewn with terms of comparable ponderosity. So, rebuffed and bewildered, I have retreated to sample two of the case histories to see how his followers have applied his methods in instances where the language and the events are more familiar to me.

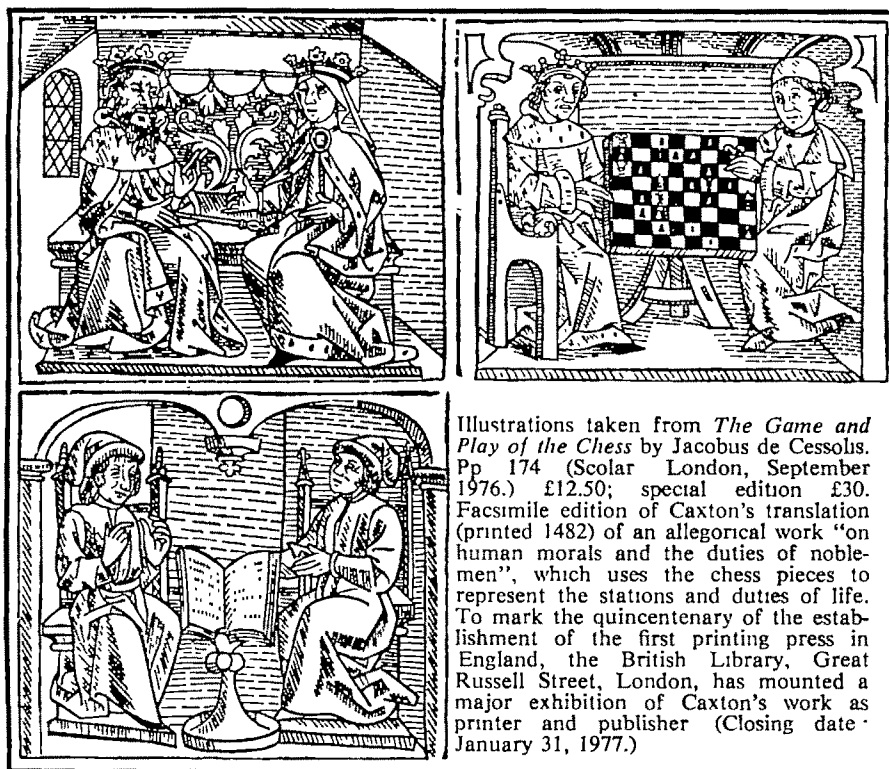
In the course of asking "Why did Einstein's programme supersede Lorentz's", Elie Zahar claims that his particular ideas of how physics may be furthered by translation into mathematics are illustrated by an account given by Peierls of the way in which Maxwell arrived at his famous equations. Although Zahar credits Peierls with this account (1963) it had appeared in various textbooks of physics long before, and—as Peierls himself took pains to state—there is no evidence in Maxwell's writings that the latter thought, consciously or subconsciously, in the way described. Briefly, the invention of the displacement current is credited to a desire by Maxwell to balance one of his equations of the electromagnetic field. But in his original (1864) paper Maxwell himself gave his

own, quite different, line of thought. He said that he assumed that the aether was a medium of "small but real density" capable of being polarised in a similar manner to a material dielectric; and that while polarisation was taking place there was a displacement of charge which "does not amount to a current because when it has attained a certain value it remains constant, but it is the beginning of a current". Peierls discounts this, by saying frankly that "Maxwell arrived at the extra term by using a picture that we do not accept today". But the question is not what Maxwell might have done if he had present-day knowledge, but by what route he in fact arrived at the outstandingly imaginative concept of a displacement current *in vacuo*. Maxwell himself recorded his approach clearly; and this was by physical analogy rather than by mathematical analysis. So, although most of us are aware that mathematics may suggest creative steps in physics, Maxwell's displacement current is not an example, and its history would have to be bent to fit Zahar's philosophy. Perhaps he is not to be blamed unduly for relying on so eminent an authority as Peierls, but he fails to quote the reservation, made by the latter himself, which completely destroys the argument.

I have to emphasise that I have only sampled Zahar's monograph and it would require much effort to treat it in detail; it has 141 footnotes and 82 references. Peter Clark's study of "Atomism Versus Thermodynamics" runs to 244 footnotes and 196 references, and John Worrall's "Thomas Young and Newtonian Optics", 252 footnotes and 83 references. Such assiduity cannot be reviewed at length, but one further sample will illustrate why to me the book is dangerous. It concerns the famous two-slit interference experiment of Thomas Young.

Worrall says: "There are, I claim, sufficiently many suspicious aspects of Young's account of the two-slit case to support the belief that he never performed the experiment"; and "However, as I have said, there is evidence that Young never performed the experiment"; and once again "Moreover, the dearth of details in Young's account makes it seem unlikely that Young ever did successfully perform it, and certain that he did not give sufficient information about the experiment to ensure its repeatability by others".

What are the facts? Worrall makes play of the point that Young did not give a full account of the experiment, which he described only briefly in his course of lectures at the Royal Institution between 1802 and 1806. Worrall says that when Young really did an experiment he usually gave enough



Illustrations taken from *The Game and Play of the Chess* by Jacobus de Cessolis. Pp 174 (Scolar London, September 1976.) £12.50; special edition £30. Facsimile edition of Caxton's translation (printed 1482) of an allegorical work "on human morals and the duties of noblemen", which uses the chess pieces to represent the stations and duties of life. To mark the quincentenary of the establishment of the first printing press in England, the British Library, Great Russell Street, London, has mounted a major exhibition of Caxton's work as printer and publisher (Closing date: January 31, 1977.)

details for others to repeat it. But there are possible explanations for Young not describing the Royal Institution experiment in detail: he was speaking to what Brougham denigrated as "an audience of fashionable ladies", and the two-slit experiment, which is described in the printed version of his Royal Institution lectures, is in no more and no less precise terms than many of the other experiments recorded in the lectures.

Further, Young may not have thought the two-slit experiment sufficiently important to be described in a fully scientific paper, because he had already described an equivalent experiment in his 1803 Bakerian lecture. On that occasion he allowed sunlight to pass through a pin-hole and had inserted a cardboard strip about one-thirtieth of an inch wide in the beam diverging from the pin-hole, so as to obscure the central portion of the beam. In the plane of the card there would be left two patches of light roughly equivalent to what would have passed through two slits with their inner edges separated by the width of the card. He then observed the interference pattern produced by the light passing through these two imaginary slits, and showed that the interference fringes disappeared if one or other was obscured. If, as his Royal Institution lecture suggested, he subsequently used two real slits, he may have thought the experimental modification so trivial that he never troubled to describe it in detail.

Now did or did not Young ever do the experiment with two actual slits? Although he described the experiment, he did not say in so many words that he had performed it, and so we cannot summarily dismiss Worrall's claim. And what I have said so far as an alternative explanation involves as much speculation on my part as the contrary involves on Worrall's. But he goes on to give physical reasons why Young would have found the experiment so difficult to do that he would surely have mentioned the difficulties. Worrall says, for example, "We know, in fact, that if the difference of the paths from the two edges of the source to either of the slits is greater than about $\lambda/4$ the fringes will not in general be observable". The implication is that the pin-hole has to be so small that there will be very little light, and that since Young said that the effects were to be observed on a white card (and not viewed in transmission by an eye-glass, as Fresnel later used) Young would have had so little light that he "never successfully performed the experiment".

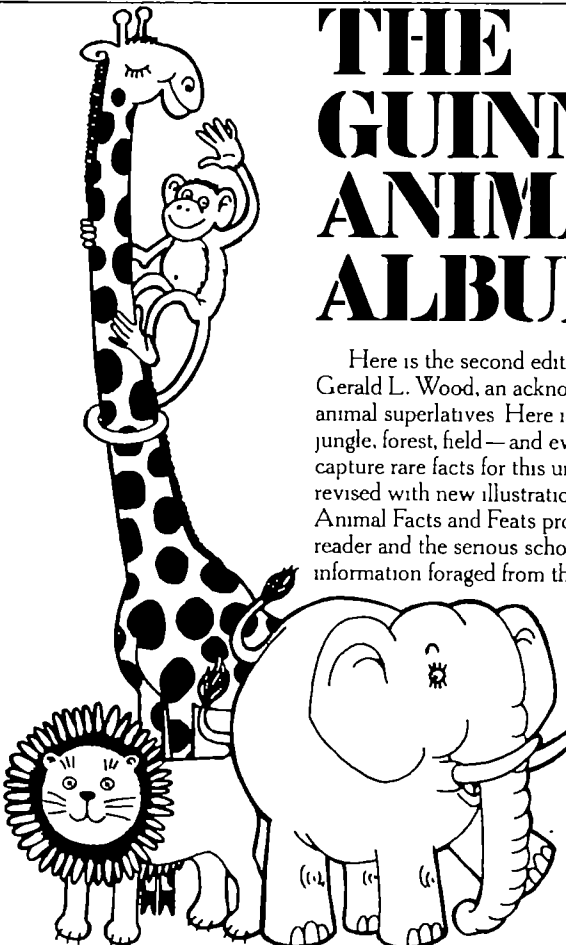
Anyone with a sense of scientific, if not historical, responsibility making such a charge against one of the great

experimenters ought surely to try the experiment himself or to ask a competent experimenter to see whether it is as difficult as he thinks, for it is a singularly easy experiment to check. It merely involves a darkened room, a pin-hole, two adjacent slits scratched in an aluminised mirror, a postcard, and the sun. Stimulated by the need for this review, Dr W. J. Bates and I have made such a check, and no more than a few minutes were necessary to show that Young could have done the experiment very easily. We made a pin-hole in a blind, and if this had had to satisfy the coherence criterion quoted by Worrall, we might indeed have had difficulty in seeing any fringes. But we found that a hole as large as 3 mm in diameter was permissible with a distance to the slits (which were 0.04 mm wide and separated by 0.4 mm) of 120 cm. The fact is that Worrall's coherence criterion, if not completely irrelevant, is far more stringent than the experiment requires—by a factor of at least 40. This makes piquant reading of Worrall's comment: "Young had no clear ideas of what was later called coherence which (had he had them) would have pointed to, and explained the necessity for, a small source at a great distance from the double slit". There are at least two other nonsenses in Worrall's exposition, and Young in

his ignorance was much better off than his twentieth century critic—with the latter's misunderstanding of physical optics. There is enough light with a 1-mm diameter hole not only to see the fringes, but also to see the familiar colours associated with white light fringes.

Young's arguments can sometimes be criticised: he believed that waves could not exert radiation pressure, although Euler had shown that they could, and that light waves were longitudinal. But we can understand why he might not have stressed the double-slit experiment with real slits, even if he had done it, because he considered it easy to do, as we found it easy to repeat from the information in his lecture. Any suggestion that he could not have done it, and did not give enough information for it to be repeated, is therefore irresponsible.

I may have been unfortunate in my sampling, but I can applaud only the last monograph in the book, by Paul Feyerabend, who concludes of the methodology of research programs: "It has not led to a better understanding of science and it is even a hindrance to such a better understanding because of its habit of beclouding facts with sermons and moralising phrases". The book is not completely worthless, in that it is a useful source of reference,



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GUINNESS SUPERLATIVES

and it is at times challenging. But it supremely illustrates the dangers that now beset the history and philosophy of science. Dionysius may have been right in regarding history as philosophy teaching through examples, and the analysis of case histories is therefore worthwhile on this account. But historians and philosophers of science are becoming increasingly involved in talking to one another in a self-propagating language and literature of

their own, and all too many have no working contact with experimental science, where there is no place for assiduous nonsense. Socrates said that philosophy was not for those without experience: and this is no less important for natural philosophy than it is for moral. □

R. V. Jones is Professor of Natural Philosophy at the University of Aberdeen, UK.

Plasticity and resilience

Freda Newcombe

Early Experience: Myth and Evidence. By A. M. Clarke and A. D. B. Clarke. Pp. x+314. (Open Books: London, 1976.) £3.95. *The Growing Brain: Childhood's Crucial Years.* By John Brierley. Pp. 138. (NFER Publishing: Windsor, 1976.) £3.55.

THESE two books reflect different facets of human growth: early plasticity and enduring resilience. The points of view are reasonable and complementary; the danger is that extracts out of context may be used to bolster overgeneralisations and dogmatic prejudice; and the encouraging aspect is that their authors are searching for the facts—physiological and behavioural—that are necessary for any advance in understanding the complexities of development.

Claims already published about *Early Experience*, ranging from the harm it may do to its "revolutionary implications for the design of education and social policy", obscure its clearly defined brief. The Clarkes have assembled evidence to support a rational hypothesis: that early experiences do not irrevocably determine future development and that "the possibilities for alteration in response to a changing environment remain open for longer than has been commonly accepted" (p18), with the reservation that "this responsiveness decreases in time" (p272). They comment on the inadequacy of received wisdom on child-rearing, they point to the discontinuities in normal development, and the rôle of learning (and—equally important—the possibility of unlearning inappropriate responses); and they note a "remarkable resiliency", a "self-righting tendency" in children, inferred from their physical, intellectual and emotional survival after early years spent in

appalling conditions of isolation and cruelty (see chapters by Davis and Koluchova). Evidence is also drawn from anthropological and animal investigations to show that "the young animal retains an enormous capacity for change in early patterns of behaviour and cognitive competence, especially if the initial environment is seriously altered" (Kagan, p121).

Group studies in the book suggest that the outcome for children initially reared in institutions is related to the adequacy of later placements in other institutions (Skeels) or adoptive families (Tizard and Rees). Children from poor backgrounds may make good progress after relatively late adoption (Kadushin) or institutional care (Lewis; Clarke and Clarke; Reiman). An interesting chapter (Bronfenbrenner) on the so-called "head-start" programs makes the point that the substantial gains shown in the first year tend to fade unless reinforced by follow-up programs and parent participation. The evidence from head-start programs is, however, two-edged. Of more direct impact is the report (Rutter) on parent-child separation, and the significance of genetic and environmental variables. It suggests that the *cause* of separation rather than separation itself may be associated with maladjustment and delinquent behaviour.

The Clarkes have not glossed over the problems of sample, selection and measurement which make quantitative generalisations about child-rearing practices untenable. They are well aware of the constraints on development imposed by constitutional factors; and they do not push their argument to the limit: "there is absolutely no implication that infancy and early childhood are unimportant, only that their long term rôle is by itself very limited" (p272). Negative data, as far as they are aware, do not invalidate a system (p272). By the same token, it is possible that observers have failed to detect subtle changes of language, intelligence, social skills, and personality in children who are at risk in the early years of life. Psychometric tests and questionnaires have their uses and their limits; "the marked

inability to concentrate of many institutional children by the age of five" (p149) is one of the minority notes in this volume that would be interesting to pursue.

It is a pity that some of the authors in the Clarkes' book were recently featured as "the anti-Bowlby team", and that their book has been described as likely to do more harm than good. In turn, the reported suggestion of one of their authors that Bowlby's influence has been "mainly bad" on middle-class mothers is equally inappropriate. It is easy to assign the rôle of spectre in the nursery (*pace* Spock), but that this has perhaps too often been done after somewhat biased interpretations of the maligned experts' texts. And both Bowlby and the present authors have made a positive contribution to the understanding of behaviour. Only those who cling obstinately to a single explanation—the either/or fallacy that clouds so much political and sociological thinking—will adopt a militant stance. The Clarkes' book will be of great interest to those psychologists, clinicians and caseworkers who are concerned with the theory and practise of child-rearing.

DR BRIERLEY'S book, addressed to parents and teachers, summarises work in neuropsychology and developmental and physiological psychology that has a possible bearing on human growth and educational needs. It reflects wide reading in rapidly expanding disciplines and takes on the challenging task of distilling this personal selection into a coherent and feasible view of human development: a brave assignment worthy of Browning's view of man's reach.

Few will quarrel with its main assumption: the child has unique genetic equipment, it is very dependent on the environmental stimulation it receives during the early years of life, and yet it is endowed with a remarkable degree of flexibility and tolerance to stress. The practical implications of this viewpoint are discussed with a plea for an environment rich and varied enough to sustain the diverse needs and talents of human creatures. In contrast, there is little stress on the need for structured training in the early years. In this context, a report in the Clarkes' book that "cognitively structured curricula produced greater gains than play-oriented nursery programs" (Bronfenbrenner, p247) is interesting (a seldom cited and somewhat unfashionable point of view, that might be worth reviving if the percentage of children in the UK with problems in reading, writing and arithmetic is to be reduced).

Given the vast scope of the book,

it would be unreasonable to cavil at details. Inevitably, the selection of experiments will not be approved by all physiological psychologists. Similarly, neuropsychologists may find the functional maps of the brain oversimplified and note that the concept of hemispheric asymmetry of function and the special rôle of the right cerebral hemisphere for *certain* aspects of visual and spatial perception were established long before the study of a small group of patients with callosal sections (p53). Linguists may well be startled by the view that "the remarkable power to speak foreign languages . . . can be explained on the basis of conditioned reflexes" (p 96); and those concerned with brain mechanisms may consider that too much stress has been placed on brain weight at the expense of circuitry and the complexities of modular organisation. The account of physiological

mechanisms and biochemical changes that may underlie the processing and storage of information in the brain is necessarily speculative; and the section on memory and learning is limited to the notion of a "hippocampal taperecorder" (p90), omitting much recent clinical and experimental work in this field.

As a general introduction to the topic, however, the book is a useful, well written and stimulating guide, remarkably free from jargon and pitched at the right level for the readers for whom it was designed. Few would do better or even have the courage to attempt it. □

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Representation of meaning

John Morton

Language and Perception. By George A. Miller and Philip N Johnson-Laird. Pp. viii+760. (Cambridge University: Cambridge, London and Melbourne, August 1976.) £12.50.

THIS book is about the representation of meaning. Tired of waiting for linguists to produce a usable semantic theory the authors have started to build a psychological one. The starting point is the perceptual world and its representation in memory. A conceptual theory of memory links this with language. On the one hand we have words and on the other we have ways of testing the perceptual world to find what concepts are appropriate to describe it. In this way we can learn and put what we see or remember into words, or take appropriate action when someone addresses us. In the latter case the sentence is translated into a program which can then be executed appropriately.

Miller and Johnson-Laird illustrate their position with the sentence "Did Lucy bring the dessert?" This is translated into a program which they represent as: find (M(EP), $F(x, y)$) This program will be interpreted as an instruction to search for a particular event in a particular (episodic) memory store. F is a pointer to a particular operator which characterised the meaning of the verb 'BRING'. x and y point to the concepts corresponding to 'LUCY' and 'DESSERT'

respectively. This translation procedure is automatic for the listener who knows English well. He then has the option of answering. This is organised by an executor, which must find the episode and apply the various tests which define BRING: (i) find (domain, COME (agent)) and call it e ; (ii) test (during e , HOLD (agent, object))

These tests can be interpreted in this situation (after the various arguments have been assigned their values) as: (i) find (and label as e) the episode when Lucy came; (ii) find out whether Lucy was then holding the dessert. The operator COME involves further tests which include various perceptual predicates concerning the motion of Lucy; HOLD is itself such a perceptual predicate.

The procedures may fail in a number of ways. the relevant episode may not be found; Lucy might have been carrying something other than dessert. These failures would be interpretable by the executor, who could try alternative ways of answering the question or organise a reply—including that of "Don't know".

They discuss some of the ways in which syntax might influence the translation program, including control instructions such as 'ACHIEVE', which result from commands. And they outline ways in which factors such as intonation, context and intent would influence the processing.

The next problem was that of exploring of lexicon in more detail, having established the kind of machinery the lexicon would be involved in. Initially they attempted to do this using purely perceptual tests in the lexical entries. But that soon became inadequate and they introduced functional information. Thus a table must have a WORK

TOP which is defined not just as being flat—readily testable perceptually—but also it must be "large enough, and rigid enough, so that, if it is supported horizontally at an appropriate height, it will support objects for a person to manipulate with his hands." Such a definition incorporates sensorimotor predicates in its evaluation and the need for analogic prediction. Consideration of speaker as well as hearer necessitated a more flexible approach to the lexicon, and the need to include in the theory some acknowledgement of the relationships between Lexical items led to the idea of core concepts in semantic fields.

The core of FURNITURE is that it assists in accommodating people's bodies and the objects and instruments they use as they engage in eating, sleeping, working, or playing. This core motivates the conditions in a decision table. The conditions are tests with YES/NO answers and the pattern of answers to the conditions leads to particular lexical concepts. Thus for SEAT there are conditions which ask whether the object (1) is for one person only, (2) has a backrest, (3) is upholstered and (4) has legs. If the first two answers are YES then the object must be a CHAIR; with the pattern YNYN it could be a footstool or an ottoman, and a further test or specification would have to be made. The advantage of the decision tables is that they can be used in both ways—from properties to lexical concepts or *vice versa*. Verbs are related together in a slightly different way in this theory, there are core concepts such as TRAVEL, POSSESS, SEE which are then refined in various ways, such as by specifying the manner—to give verbs such as LURCH, OWN and GLIMPSE.

The semantics is not just of language but of the real world as we perceive it; objects, spatial relations, actions, time; and the use of psychological data is more apparent on the perceptual side of their theory than on the language side. The authors seem to make the Whorfian decision to base perception on language—in this case English. All words are related to the perceptual world and all aspects of the perceptual world are related directly to the lexicon-based conceptual structure. What they do not do is create an intermediate level. The decision tables seem to be language specific, and it is not clear how we can think other than verbally. To take a simple example: I feel I can conceptualise separately. we (me, you and him); we (me and you); and we (me and him). The decision table, however, produces only the conditions—the tests of who is included—and the words. The three versions of *we* are separated only in the columns

of the decision table, which are not the right kinds of things to serve as cognitive units. Their decision may have been the right one but I would have liked to see their motivation.

I expect there are going to be lots of flaws in the routines they suggest. I didn't specially look for them but some jumped out at me—such as the requirement, in the definition of LOOK AT, that the back of the eyes, the pupil and the object looked at have to be in line. Not only can you look at someone through a mirror but also out of the corner of one's eye. The former objection may be trivial but the latter looks as though it will require a distinction to be made between the routines used by the looker and by the looked at. This is a game the authors couldn't hope to win first time out. Either they analyse a few concepts into the ground and produce something lacking generality or they attempt to cover enough ground to justify general principles with the risk of getting details wrong.

While their local arguments are coherent, the book as a mass is rather heavy. I get the feeling that they were looking over their shoulders at linguists and philosophers, and in so doing blurred their psychological position. Their final 'Conclusions' chapter, which might have clarified this, reflects rather the "thank god that's over" feeling of the Preface. One little thing which made the book more difficult to read than it need be is the idiosyncratic indexing. They construct lexical entries for scores of words but don't include the words explicitly in the index. As it is, PERSUADE can be found through 'Communication verbs: perlocutionary'; and core concepts in their model, such as 'core concept', have no entry at all.

To obtain a rounded and more leisurely evaluation of this book (without actually reading it, that is) you will have to watch out for reviews in linguistic, philosophical and psychological journals. Each will have different objectives, requirements and criteria, and together they will pick it over as thoroughly as it deserves. At the moment I have no idea how many of the authors' ideas will survive, but I am certain that it will provide a fresh impetus to work in the area. For whatever its technical weaknesses may be, the outstanding merit of the book is to show how difficult the task is by submersion rather than dipping in the big toe. People really interested in the area have no option but to look at it.

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Biographical springboard

June Goodfield

Half a Century of Medical Research. Vol. 2: The Program of the Medical Research Council, UK. By A. Landsborough Thompson. (HMSO: London, August 1976.) £10.

THIS is the second of two volumes which together cover half a century of research effort, and form a history of Britain's remarkable and distinguished institution—The Medical Research Council (MRC). The first volume (for review, see *Nature*, 252, 134, 1974) covered the constitutional and organisational aspects of the Council's history and dealt with the intriguing issues of the origins of policy and, "the formulation of those administrative principles" that related to the promotion of medical research. It could have been—should have been—a fascinating book: in the event it is impeccably dull.

This second volume is concerned with the Council's scientific program in detail: once again with the origin of policy and the varied circumstances which led to certain subjects being selected for special support. But now the context is broader for as the MRC itself was expanding over the sixty years in question, so too were the boundaries of the scientific world. So the Council's efforts and achievements must be assessed in terms of the wider international effort in biomedical research.

By any standards the record is most impressive. For measured either by an intuitive feel for fields likely to be significant, or by a balanced stance that recognised the legitimate interests of both the government and the scientific community, serving the one without stifling the other, the record is, on the whole, superb. One can be thankful that, in 1913, the committee that submitted a general scheme of research ignored the narrow interpretation of the phrase—"provision of research"—laid down by considered legal opinion. The National Insurance Act of 1911 appropriated one penny per annum per head of the insured population of Britain, to be allocated from parliamentary funds for "the purpose of research", and the lawyers held that research might well have to be restricted to the diseases of insured persons only. At that time, tuberculosis certainly, but possibly not cancer! But the committee quietly by-passed that

issue and we may thank God for that, for stemming from their general injunction to cover "all researches bearing on health and disease, whether or not such researches have any direct . . . bearing on any particular disease", has flowed such specifics as penicillin, the structure of DNA and the basic mechanisms of cell-mediated immunity.

One must accept that this is essentially a work of compilation. By far the greatest source of material used by the author has been the Council's own annual reports and a variety of scientific papers, reports and documents. These are bound to be bland, objective and reasonably free of human virtue, or vice, alike. So as with the first volume, this is not history as historians know it, for the human content has once more been either ignored or extracted. What has been provided is a valuable series of reference points from which others may start more vital histories.

Nevertheless, there are fascinating insights to be discerned for those who are prepared to weave a passage through the maze of detail. The social picture of Britain we have derived from other sources is intensified by a knowledge of where the early clinical efforts were directed—on tuberculosis, rickets, the hygiene regulations for milk—problems that later society does not face, either because of new knowledge or changed habits.

In the days of quack cancer cures it is salutary to be reminded once again of the human traits of venality and fear; much trouble stemmed in the early days from those who promoted dubious claims for tuberculosis. Then, as now, they "stimulated pressure groups of busybodies, and patients,

anxious to pioneer against the alleged tyranny of the professional closed shop". To set this into the contemporary situation, simply substitute "cancer" for "tuberculosis", "lactrile" from apricot pits, for a "powder with a resolute Zulu name extracted from the root of a South African plant", and the "contemporary medical profession together with the US Food and Drug Administration", for a "professional closed shop".

Even Dr Summerlin and the affair of the painted mouse had its counterpart within the Institute's hallowed walls, for in 1927, virology revealed a seamy side. One investigator claimed to have discovered a virus as the causative agent for disseminated sclerosis; she even prepared a vaccine from cultures which was actually clinically used by certain physicians, even before other scientists had tried to repeat the results. When the results could not be reproduced the scientist was invited to repeat her work under supervision. She then "withdrew from the scene", as the author coyly writes, and it was discovered that she had been using the virus of bovine pleuropneumonia which had been described by the National Institute, and from where she had obtained her cultures.

Plus ça change . . . but things are never quite the same. Science in its achievements has progressed wonderfully even if human beings seem to be much the same. But they are deeply fascinating nevertheless, and I miss them in this volume, just as much as I did in the first. □

June Goodfield is a Visiting Professor at The Rockefeller University, New York.

Fact, fiction and fraud

Michael Stoker

The Patchwork Mouse: Politics and Intrigue in the Campaign to Conquer Cancer. By Joseph Hixson. Pp. x+228. (Anchor/Doubleday: Garden City, New York, 1976). \$7.95.

IN this book Joseph Hixson tells the story of Dr William Summerlin, and much more besides. Summerlin, a dermatologist turned immunologist, claimed that tissue which had been kept in culture could be grafted across transplantation, even species, barriers. But, in 1974, when working at the Sloan-Kettering Institute in New York, he



was caught red (or rather black) handed after using a felt pen to touch up some supposedly successful pigmented skin grafts in white mice. The subsequent enquiry revealed other evidence or de-



Robert Good

liberate misrepresentation to his colleagues. For example, untouched rabbit's eyes had been shown at several meetings as successful corneal transplants.

A nasty little episode no doubt, and pretty awful for those involved, but why should a distinguished science writer bother to write a book about it? After all it is not the first time that a scientist has been caught cheating; and, paradoxically, where fiddling of evidence is the greatest sin, as in scientific research, it also has the least consequence in the long run, since the cheat destroys himself, not science.

The Summerlin incident had special features, however, which must have proved irresistible to Hixson, and which he has exploited with skill and flair. First, the claim itself was of tremendous potential importance, not only for patients waiting for grafts, but for its bearing on immunological theory, based as it is on the classical concepts of Burnet and Medawar. All this is painlessly explained in considerable detail and with the expertise of the good professional journalist. Second, Summerlin, not normally one for rushing into print with detailed articles in scientific journals, is apparently a charming and persuasive speaker, and his transplantation story had received the full treatment from the press and radio networks. Small wonder that the media were not silent when the science writers found they had been caught out. Hixson's often riotous, but always sympathetic, accounts of his colleagues in the press corps are not to be missed, if only for the educational content, which should be recommended reading for graduate students.

Most important, however, in Hixson's view, was Summerlin's relationship with Dr Robert Good, Director of the Sloan-Kettering Research Institute, the largest cancer research establishment in the United States, one of the original triumvirate advising the

President on the great cancer programme and one of the most powerful figures in immunology and cancer research. Good had been intrigued by Summerlin's claims, and brought him to New York in an exalted position, apparently to the disquiet of some of the original senior staff of the Institute, who were inevitably grumbling about a new Director riding roughshod over the existing regime, and pushing on his own ideas, especially on tumour immunology, to the forefront. Good, although a co-author with Summerlin (presumably as his sponsor) had never been directly involved in the work himself, and partly as a result of reports from Summerlin's own staff, had indeed begun to suspect something fishy some months before the ink episode. Summerlin's defence was that he broke down in the face of Good's insatiable demand for significant data (they were certainly needed); and instead of being summarily sacked, as many might believe should follow such dishonesty, he was actually rewarded with one year of terminal sick leave.

The pressures in the Sloan-Kettering, and its special position, thus leads Hixson inevitably to the uneasy power politics of the proposed (and ordained) 'Conquest of Cancer'. Although it is all dealt with historically, and with little explicit statement of the author's personal view, the message which emerges in Hixson's book is clear—that vote-seeking congressmen, anxious administrators and a hungry press, are tempting greedy scientists into exaggerated predictions and claims about cancer, which if not actually as fraudulent as those of Summerlin, may seem so in retrospect to relatives of cancer patients.

The Patchwork Mouse is not written for the usual *Nature* reader, and you have to swallow a colourful narrative style, which may not always be based on direct observation. "Robert Good has shaved and pulled a white turtle neck shirt over his dark, tousled head at an hour when the swinging East Side singles bars have barely closed their doors". How does he know? Perhaps Good gets into his turtle neck feet first. There are also a few errors. For example, cancer as a whole is not commoner in those with defective immunity, only certain rare forms of the disease. The text seems, however, to be generally accurate with careful qualification of statements and quotations whenever required.

This is a fascinating and readable story with compassion for the people involved. Unlike the principal character, Hixson tells it as it is. □

Michael Stoker is Director of Research at the Imperial Cancer Research Fund Laboratories, London, UK.

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Memorial to cotton research

H. S. Darling

Agricultural Research for Development: The Namulonge Contribution. Edited by M. H. Arnold. Pp. x+353. (Cambridge University: Cambridge, London and New York, August 1976.) £13.

THE Cotton Research Corporation (CRC) was established by Royal Charter in 1921 under its former title of the Empire Cotton-Growing Corporation. For 55 years until it was dissolved in 1976, the CRC played a leading role in research and development in support of the field production of the cotton crop in the anglophone countries of the tropics especially in Africa. From its administrative headquarters in London the CRC maintained a team of thirty to forty carefully selected and trained research workers distributed around the world and renowned alike for their unity of purpose and the consistent high quality of their work. The CRC team was regarded with considerable justification as a *corps d'élite* among the British agricultural scientists who have made such an important contribution to the development of the anglophone territories of the Third World.

The deployment of CRC staff was governed from the outset by two considerations. The first was the need to provide research assistance, often of a trouble-shooting nature, in areas in which increased cotton production was desired. In such cases, CRC workers were posted to government agricultural stations in cotton-growing areas to give specialist support to local research programmes. The second consideration was the need to maintain at least one permanent multidisciplinary research centre under direct CRC control, where long-term work of a fundamental nature could be carried out on problems peculiar to the cotton crop with the object of providing information of relevance throughout the tropics. From 1945 to 1972 this second need was met by the Namulonge Cotton Research Station in Uganda.

As its subtitle indicates, the book under review is concerned with Namulonge and is a composite production from the pens of senior scientists, each a specialist in his own field, formerly on the staff there. Editorial unity has been gently imposed by Dr M. H.

Arnold, the last CRC Director of the station, who is himself a major contributor. The whole provides an excellent description of Namulonge and of its work over the period 1945-72. Sited in the centre of the southern half of Uganda some 35 km north of Kampala, the station covered 900 hectares of reasonably fertile soil, most of which was suitable for field trials and other forms of agricultural research. Established on land leased by the CRC from a Ugandan landlord and built with UK funds, the station was well equipped with laboratories, other research facilities and housing for staff. In 1972 the station was handed over to the Ugandan Government as a going concern some three years before the CRC itself came to the end of its active life.

The meat of the book lies in carefully written yet readable descriptions of research at Namulonge given in chapters 2-8 and comprising two-thirds of the total text. These seven chapters bear the following titles: agrometeorology; soil productivity; crop physiology; entomology; plant pathology; resistance breeding; and plant breeding. They are followed by a chapter of importance on Namulonge farm and a closing chapter on the application of agricultural science to national development. There is also a references and author index and a full subject index.

The accounts of research are always competent and often brilliant. The descriptions of cotton-breeding in chapters 7 and 8 are highly authoritative, embodying as they do cumulative experience of 40 years of work by several generations of CRC geneticists and breeders including men of the standing of J. B. Hutchinson and R. L. Knight. Surprisingly enough, the name of S. C. Harland is conspicuous by its absence! The chapter on Entomology is to be commended for its clear and accurate analyses of complex situations resulting from the sequential incidence of a wide range of pests, and from the interaction of the effects of insect-damage and planting times. Less satisfactory is the chapter on soils and soil productivity where no mention is made of work in Nigeria by B. W. Bache, P. R. Goldsworthy, R. G. Heathcote, D. Lawes and others, which presumably was well known to the Namulonge team. This West African research preceded and paved the way for the agronomic advances described by the Ugandan workers in the late 1960s and early 1970s.

For all its general scientific competence and its areas of technical brilliance this book is less than satisfactory in that it fails to ask, let alone seek to answer, at least three important questions. First: why was Namulonge

chosen as a site for basic research on cotton-growing? Second: why does no thought seem to have been given in the planning of work programs to research on the human factors that dominate cotton production? Third: why is so little mention made of plans for training local research workers to replace European staff when (as has already happened) CRC control came to an end? These questions are of major significance.

The choice of Namulonge was made when the reviewer was working as an agricultural research worker in the then Ugandan Protectorate Department of Agriculture. It seemed obvious that the site was atypical of areas suited to cotton production in that it was too cold and was unsatisfactory in respect of rainfall distribution. It has long since been accepted by Ugandan farmers that the area is agronomically suited to the growth of robusta coffee and plantains rather than cotton. The farming systems laboriously worked out by the Namulonge staff, although successful in themselves for making the best of cotton-growing in local conditions, are largely irrelevant to local agriculture and are to that extent a waste of effort.

Successful cotton-growing in the near-subsistence conditions that obtain in Ugandan agriculture, and indeed throughout much of tropical Africa, depends on plentiful supplies of human labour. Any research program aimed at increasing cotton production in developing countries should recognise the importance of human factors and, where these factors are not clearly understood, should seek to define and elucidate them. Technical developments conceived without relevance to human considerations often fail to be adopted. A classic example of this is the question of planting dates for cotton in Uganda. Here scientific evidence indicated that early planting was essential for best results in terms of yield. All human experience showed that local farmers found such early planting to be impracticable in the context of their overall farming systems. In the view of the British research worker, trained in the natural sciences, it was essential that early planting be advocated as official policy and the resulting impasse has dominated local cotton-growing policy for many years!

Namulonge, in common with many other British-based agricultural research stations, failed to appreciate the need for research inputs from economists and rural sociologists in the interdisciplinary mix of the station program as a whole. The failure is apparent in the last chapter of the book on the application of agricultural science to national development, which is too vague to attract attention or

inspire confidence. The workers at Namulonge clearly felt confident of their ability to advise on growing cotton. It is equally clear that they had few ideas as to the role of cotton production in the Ugandan economy.

The apparent lack of attention to the training of African staff is surprising. The upgrading in 1948 of Makerere College at Kampala to university status enjoying special relationship with London University followed closely on the establishment of Namulonge in 1945. The first director at Namulonge, Sir Joseph Hutchinson, was Chairman of the Council of Makerere College from 1953 to 1957, and played an important role in the development of the excellent Faculty of Agriculture there, including the establishment of its outstation at Kabanyolo. Namulonge's staff were

encouraged to lecture in the Faculty. Yet in spite of these close ties between Namulonge and Makerere, little or no thought seems to have been given to using the new university to build up a body of local scientific expertise in cotton research.

This grave omission was unfortunately a major weakness of the CRC as a whole. The dissolution of the Corporation in 1976 has consequently meant that with the disappearance of the London headquarters, little in the way of research staff and human assets persist in a recognisable form. A proud ship has sunk virtually without trace and the book under review can almost be regarded as its memorial tablet. □

Dr Darling is Principal of Wye College, University of London, UK.

Self-reliant development

C. H. G. Oldham

Beginnings of Brazilian Science: Oswaldo Cruz, Medical Research and Policy, 1890-1920. By Nancy Stepan. Pp. xi+225. (Science History: New York, June 1976.) \$12.95.

EFFORTS to build up local research capabilities in many developing countries over the past two decades have frequently been frustrated by the lack of local demand for the results of this research. As a result, policy makers in these countries are currently preoccupied with measures to stimulate demand, and to make science more relevant to national needs. This is proving to be no easy task since the science system has grown in isolation from the productive system and the forging of links is exceedingly difficult.

Nancy Stepan's excellent book, *The Beginnings of Brazilian Science*, shows how governments in the developing world might have been saved a good deal of unnecessary expense and anguish had they known of, and heeded the lessons which were to be learnt from the growth of biomedical science in the late nineteenth and early twentieth centuries in Brazil.

Most of Professor Stepan's book, and certainly the best part of it, is devoted to a case study of the Oswaldo Cruz Institute in Rio de Janeiro. This Institute was initially established in 1900 to produce yellow fever serum but grew into an excellent scientific centre covering the entire spectrum of scien-



Oswaldo Cruz

tific activities—basic research, development, staff training and even production. It had an international reputation and was staffed entirely by Brazilian scientists. Those looking for an example of self-reliant development in science in the third world would have to search hard to find a better example than the Oswaldo Cruz Institute. To emphasise the positive lessons which she thinks are to be learnt from the history of this Institute, Professor Stepan contrasts its success with the failure of the Bacteriological Institute of Sao Paulo, established at roughly the same time. The story of the Sao Paulo Institute, although useful for comparative purposes, palls in comparison with the exciting and dramatic success of its Rio de Janeiro rival.

Professor Stepan clearly shows that the success of the Oswaldo Cruz Institute owes a great deal to the happy coincidence of the right people being brought together at the right time and the right place to meet a particular need. The need was particularly urgent. Brazil, at the turn of the century, had

most of the physical ingredients required for the development of a prosperous and wealthy nation. The main drawback was its extremely unhealthy climate. Rio de Janeiro was largely avoided by foreign visitors because of its foul reputation for disease. Epidemics of yellow fever, bubonic plague and other tropical diseases caused the deaths of thousands of people each year. The need for a solution to these epidemics was apparent to the public and politicians alike. There was a 'demand' for the results of scientific research.

Fortunately the time was also ripe scientifically for a biomedical research institute established in a developing country to be able to make internationally significant contributions, and at the same time solve a pressing national need. Had the politicians provided the funds thirty years earlier, it would have been too soon. But, in the latter part of the nineteenth century, due in large measure to the work of the Pasteur Institute in Paris, an understanding of the bacteriological origins of many diseases had been developed and the whole field of immunology had been opened up. By 1900, when the Institute was founded, there was a real opportunity for major advances to be made in bacteriology; an opportunity which the Brazilian scientists were not slow to exploit not only for the benefit of the Brazilian people, but also for the benefit of science as a whole.

The real hero of the story is Oswaldo Cruz himself. He was trained as a doctor and went to study at the Pasteur Institute in Paris. He returned to Brazil and, against very considerable political and public opposition, converted an Institute initially established solely to manufacture serum, into one of the world's leading biomedical centres. His approach was almost the exact opposite of the one normally taken by developing countries today. Instead of starting with research and then trying to find some use for the results, Cruz began with a need, and with a solution to a part of that need—the manufacture of yellow fever serum. He then worked from this to applied research and eventually basic research. This distinction between basic and applied research, however, does not seem to have been one which bothered those Brazilian scientists working at the Oswaldo Cruz Institute. For them there was a job to be done, and if this job required advancing the frontiers of science, this was part and parcel of the job. If on the other hand all that was required was some simple adaptive work in development, then this too was carried out. The Sao Paulo Institute never developed the same range of scientific

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activities and, according to Nancy Stepan's analysis, suffered as a consequence.

The book is a fascinating story of the interplay between science, politics and development as they relate to the growth and utilisation of the biomedical sciences in Brazil in the first three decades of the twentieth century. It is marred only by an over-ambitious title (the book only treats some aspects of the biomedical sciences, and not, as it implies, science as a whole), and by an over-simplistic final chapter on the science policy problems of developing countries. There are lessons to be learned from the experience of successful and unsuccessful institutions, and Professor Stepan is right in suggesting

that some of the lessons to be learned from the Oswaldo Cruz Institute are as valid today as they were in the early twentieth century. The times, international relationships, and local political situations have all changed, and Nancy Stepan probably exaggerates the extent to which the lessons have relevance for policy makers today. Nevertheless it is a good book, well researched, and is highly recommended to anyone interested in the broader policy implications of biomedical research, public health, Brazil, and development. □

Dr Oldham is Deputy Director of the Science Policy Research Unit, University of Sussex, UK.

Horological insights

A. A. Treherne

The Lost Science of John "Longitude" Harrison. By W. S. Laycock. Pp.159. (Brant: Ashford, Kent, 1976.) £18.75; \$45.75.

It is fitting that in this year, which marks the bicentenary of John Harrison's death, a book should be published which establishes clearly his contribution to science, and in particular to the science of horology. Even if the reader cannot follow the author in believing that Harrison's contributions to the science (as distinct from the practice) of horology surpass those of even Huygens, the author does establish beyond reasonable doubt that Harrison deserves more than the standard, cursory mention in the physics textbooks for his grid-iron pendulum—a remarkable achievement in itself which is, perhaps, for the first time adequately treated by the present author.

Harrison's contemporaries could never quite bring themselves to believe that a man without the standard academic credentials or any formal horological training could make significant contributions to horological science. Hence the reluctance of the Board of Longitude to award to Harrison the full £20,000 prize offered by the British Government for a solution to the problem of determining longitude at sea, even though during the sea trial of 1761 his 'sea clock' performed well within the limits laid down. Harrison's unorthodox ideas seemed perverse or even absurd in the light of the established wisdom of his time, and in spite of his practical successes few made any attempt to understand the principles behind his timekeepers,



John Harrison

Photo: Science Museum, London, UK

ignoring his published writings as the polemical abuse of a frustrated man. Later historians of science have even less excuse than Harrison's contemporaries for not recognising his successes as arising from the application of scientific method and principles, and for not seeing his work as a clear and natural continuation of the work of Huygens. It is refreshing to find the case so well argued in this book.

The unconventional methods which Harrison adopts are convincingly analysed and justified by the author, again perhaps for the first time. The interdependence of his ideas, and the important role of his remarkable achievement in completely eliminating the need for lubricants, emerge especially clearly; underlining the impossibility of making sense of these ideas if they are considered in isolation, as they often have been in the past. In fact the author not only argues that there are good scientific grounds for Harrison's apparently fantastic claim that it would be possible to make a clock according to his principles with a rate of 2 or 3 yr^{-1} , he also outlines a programme, already begun, to construct a pair of regulators based on Harrison's ideas, in order to put the claim to the test.

Harrison's writings are by no means easy to interpret, as is witnessed by the

fact that no convincing interpretation had previously been offered. What we have in this book is an ingenious but coherent interpretation which, in spite of the necessity for a good deal of imaginative reconstruction and speculation, is, in broad terms, convincing. Moreover, the analysis is always stimulating and could hardly fail to reveal many new insights. I have two main regrets. First, the exorbitant price of the book. At this price one might have expected facsimile reproductions or transcriptions of Harrison's texts as well, which would largely have nullified my second concern: that it is not always easy or even possible to tell from the book alone what is in Harrison's texts and what is speculation or

reconstruction. There are some mistakes and misprints, which most readers will have no trouble in spotting and correcting, but which would prove confusing to a reader with little scientific background.

This book is an important contribution to horology and to the history of science. The general reader, who will require only a very elementary background in mathematics and physics to follow the arguments, will also find it fascinating and stimulating. If it does not sell well, it will be because of the price rather than the content. □

Alan Treherne is a Lecturer in Mathematics and Philosophy at the University of Keele, UK.

Ecology of plankton

G. E. Fogg

Marine Plankton Ecology. By Paul Bougis. Pp. ix+355. North Holland: Amsterdam and Oxford; American Elsevier: New York, 1976.) Dfl.130; \$49.95.

In some ways the ecology of plankton may be simpler than that of terrestrial communities. For most purposes there is only one phase to deal with and the organisms are generally smaller and less complicated than those on land. But apart from the inconvenience, expense and limitations of working at sea, the marine plankton ecologist has the difficulties of investigating events, following each other much more rapidly than in terrestrial situations and in an ever-shifting medium, which may be directly related to others that have taken place thousands of miles away. It is only comparatively recently that the resources needed to deal, even inadequately, with this situation have become available and the writers of textbooks are only just beginning to catch up.

Professor Bougis, who is Director of the Zoological Station at Villefranche sur Mer, has, however, produced an admirable and comprehensive account of this rapidly developing subject. He deals with phytoplankton and zooplankton equally, and with each he begins with a necessarily brief survey of the systematics of the amazing variety of organisms concerned.

For the phytoplankton he devotes chapters to the factors affecting photosynthesis, the nitrogen and phosphorus cycles, other nutrient elements and organic growth factors, enumeration and periodicity, and primary produc-

tivity. For the zooplankton he gives accounts of quantitative studies and distribution, vertical distribution and diurnal migration, nutrition, metabolism and energy conversion, and secondary production.

A final chapter considers the place of plankton in the marine ecosystem, touching on its economic importance and possible exploitation. Appendices deal with such diverse topics as the derivation of the Sverdrup equation defining the conditions required for the spring outburst of phytoplankton and the design and construction of plankton nets—information which is not readily obtainable from other textbooks.

With such a broad canvas it is inevitable that some areas should not be as well covered as others and one such is the topic of phytoplankton buoyancy, which is treated simply as a matter of cells remaining afloat in the illuminated surface waters. The possible value of sinking as a means of increasing nutrient uptake, its interaction with wind-induced Langmuir circulation (which is mentioned in relation to zooplankton distribution only), and the possible role of differential rates of sedimentation in determining species succession, are not considered.

The book is well illustrated and the translator has done an excellent job. There is, however, the increasingly common mistake about the plural of flagellum; fluorescence and luminescence are confused; and a unicellular film of ferric hydroxide is referred to when, presumably, a monomolecular film was in mind. There are also quite a few errors that have escaped the proof-reader. Nevertheless, these are minor matters and the book will undoubtedly be most useful to students, teachers and research workers in marine biology who have access to a library that can afford to buy it. □

G. E. Fogg, FRS, is Professor of Marine Biology at University College, North Wales, Bangor, UK.

Adaptation to Environment

Essays on the Physiology of Marine Animals

Edited by

R C NEWELL

University of Odense, Denmark

This book discusses in detail the special adaptations of marine organisms to the particular environmental conditions which they are likely to encounter in the natural habitat.

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H SMITH

University of Nottingham

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Too little, too late

H. I. S. Thirlaway

Nuclear Explosions and Earthquakes: The Parted Veil. By Bruce A. Bolt. Pp. xxi+309. (Freeman: San Francisco and Reading, March 1976.) £6.

AFTER the Soviets had unexpectedly broken the moratorium on nuclear tests in 1961, the distinguished owner of this journal (Harold Macmillan) was said to have asked his advisors to show cause why his Government should continue to support seismological research for a test ban; after all was not the UK also free of serious earthquakes? Happily the advice was convincing, the work continued and, according to Professor Bolt, to some effect in a country which had lost its impetus for experimental seismology after John Milne and his contemporaries.

I was reminded of this anecdote by the author's surprise that seismology in aseismic Sweden should be so well supported. It is good that it is because, like the Shields which are seismologically exploited by the Commonwealth countries, Sweden is as near perfect a receptor for seismic waves as one could hope to find—Uppsala was well known for its overestimates of the seismic yields of underground explosions—and in spite of such sites the technical reason why there is no ban on nuclear tests is because the threshold of seismic detection is still rather too high to deter cheating. The fact that places where the seismic transmission properties and ambient noise might be good enough for seismic monitoring of a comprehensive ban on tests are unlikely to be found is not made too clear by Professor Bolt. This means that as long as the possibility of secret tests is a cause for concern, previously unacceptable threshold treaties, like that of the new Soviet-American agreement, must suffice. The author concludes: "the veil across the future of history of civilisation has not been parted".

Nonetheless, seismologists were given every encouragement and opportunity to make a significant contribution to the unveiling; and in describing how they failed this new book by Professor Bolt gives the lay reader a broad and well balanced insight on a many-sided problem.

The basic mechanisms and effects of nuclear explosions and earthquakes, the

way in which the Earth's interior structure and the seismograph modifies the relatively simple seismic wave radiations from these sources, are explained in the first four chapters. We are given an inkling of the confusion which is to follow, but at first all seemed straightforward to the geophysicists from East and West who gathered in Geneva in the summer of 1958 to examine the possibility of monitoring compliance with a treaty to ban nuclear tests. Ironically, the weapon tests which were to deny some of their predictions had already been planned for the autumn; only a month or two after the publication of their report describing how 5 kton or more could be reliably monitored, American seismologists experimentally demonstrated that there would be a great deal of doubt about underground explosions of as much as 20 kton and a few months after that, American physicists showed (theoretically) how to convert a megaton to a few seismic kilotons by detonating in a large enough hole in the ground. That it had to be an unbelievably large hole was irrelevant; the damage was done, in the next three years the opportunity to change the course of affairs (whether for good is another question) passed and the ensuing decade has been devoted to the founding of forensic seismology.

In the end it has turned out that the 1958 experts were not so far wrong, on average, in their 'guesstimate' of 5 kton; seismic waves from yields of this order (as reported by the Soviets) have been recorded on western seismographs with disarming clarity. The trouble is that signals from Nevada are not so well received and it is on Nevada Test Site experience that the Earth is calibrated in terms of seismic magnitude to kilotons of explosive. Geophysicists have been slow to accept that the property of the upper mantle to absorb seismic wave energy varies widely according to the tectonic history of the region. For policing a test ban, it is the behaviour of explosions in regions of high attenuation which determines the threshold at which compliance with a test ban can be guaranteed.

Together with an overestimate in the number of earthquakes of a given size to be expected each year (an error which was rectified in a little remembered US Atomic Energy Commission announcement in July 1962), the existence of unknown, highly attenuating structures was the fundamental source of the early confusion. Professor Bolt takes the next three chapters to describe the results, in the course of which he outlines the contributions of the USA, the Soviet Union and the UK, and of Australia, Canada, China,

France, Japan and Sweden. The Berkner Panel Report outlined the needs in the USA and a curiosity of this report is that its principal contributor was John Gerrard, a product of Professor Bullard's Cambridge department which was to play a useful role in the UK's own contribution.

At the time Bolt completed his book, the prime importance of lateral variations in the upper mantle had not been established, nor had seismogram modelling and the refined aspects of broad band seismology been confirmed as techniques for discriminating between seismic sources and for revealing some methods for testing in secrecy; it says much for the still vigorous development of forensic seismology that Professor Bolt's book is already out of date.

The last three chapters are devoted to accounts of spin-off from the development of nuclear weapons. These include: nuclear engineering for which Professor Bolt is an enthusiast in the face of counter arguments that it is not realistic to seek test bans and non-proliferation of nuclear weapons with, at the same time, freedom to engineer with nuclear explosions, the environmental consequences on people, animals and on earthquakes, the controversies about which were initiated by the megaton sized tests in Nevada and Amchitka Island and, finally, the discoveries about the interior of the Earth which have been made possible because the hypocentre, origin time and size (all unknown quantities for earthquakes) can be measured with great accuracy for nuclear explosions. These discoveries are grouped in the chapter on seismology and nuclear politics on the dubious grounds that politics prevents the free exploitation of large explosions in the interests of seismology.

The book is equipped with appendices giving incomplete lists of nuclear tests by the USA, the Soviet Union, the UK, France and China, and the full text of the 1963 Test Ban Treaty. It is prefaced by a helpful chronology on the principal nuclear and seismological events beginning with Trinity (the first test of all) and ending with the first underground test by France in the south-west Pacific. To students of the test ban problem this French test may represent another gain for the 1963 treaty in spite of non-adherence to it by France. Professor Bolt uses boxes to enclose the more technical sidelines and thereby neatly maintains the well written flow of the text without interruption. □

Dr Thirlaway is Head of the Seismological Research Group at the Ministry of Defence Atomic Weapons Research Establishment, Blacknest, UK.

Oil shale

R. C. Selley

Oil Shale. (Developments in Petroleum Science, 5.) Edited by Teh Fu Yen and George V. Chilingarian. Pp. xi+292. (Elsevier Scientific: Amsterdam, Oxford and New York, 1976.) Dfl.90; \$34.75.

OIL SHALE is a fine-grained organic-rich sedimentary rock which releases crude oil when heated. The industrial extraction of oil from shale began in Scotland in 1862, but at the present time the oil shale industry is moribund in the UK. (A review of British oil shales has been given in a recent paper published by the Department of Energy.)

Over much of the Western World the oil shale industry was important in the past, and will be so in the future, but is dormant at the present time. It is currently practised, however, in the USSR and China. One is reminded of the White Queen's rule. 'Jam tomorrow and jam yesterday-but never jam today' (Carroll, *Alice through the Looking Glass*, Macmillan, 1871).

The world's resources of oil shale may contain some 30×10^{12} barrels of oil, only 2% of which is available for present-day commercial exploitation. This figure is controlled by two factors: the price of crude oil and extraction technology.

The conventional method of extraction necessitates mining the shale and distilling off its oil by retorting above ground. Subsurface *in situ* retorting, aided by explosive fracturing and fluid injection, is still in the experimental stage. So too are biochemical extraction methods currently under investigation at the University of California.

Due to recent advances in organic geochemistry the composition and molecular structure of oil shale hydrocarbons are well known. Similarly the geographical distribution and the geological factors which control the formation of oil shales are well documented. All that is needed to revive the oil shale industry is either a further increase in the price of crude oil, or a major breakthrough in the technology of extracting oil from oil shales.

It is a cliché of our time to say that oil is too precious to burn. It should be used only as the foundation of the petrochemical industry, never as an energy resource. Nonetheless, if nuclear, geothermal or any of the other energy sources currently under review fail to supply the demand then oil shales may become very important.

Oil Shale provides a useful review

of current knowledge of many aspects of oil shales. The book consists of twelve chapters edited by T. F. Yen, Associate Professor in the Department of Chemical Engineering in the University of Southern California, and G. V. Chilingarian, Professor, His Imperial Majesty Shahanshah Arya Mehr Chair of Petroleum Engineering, in the same university.

It is true to say that the University of Southern California is the intellectual font of the book, providing a third of the sixteen contributing authors. The geological core of the book is undoubtedly the Green River oil shale, which, according to the index, is mentioned on 66 pages of the 266 pages of text.

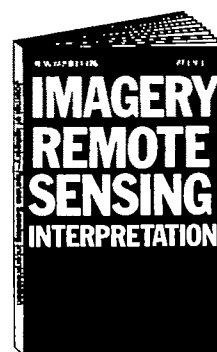
Nonetheless, the book covers the field well. It begins with a brief review of the topic. This is followed by an account of the geological setting of oil shales and their geographical distribution. The latter is displayed in a series of maps showing the distribution of oil shales during different geological periods. This could have been greatly improved had the data also been tabulated so that the reader could find the literature source for every oil shale located on the maps.

An account of the origin and formation of oil shale follows, and is succeeded by a chapter on the origin and characteristics of the Green River oil shale. This Tertiary lagoonal deposit covers large areas of the states of Colorado, Utah and Wyoming, and contains some 80×10^{12} barrels of oil (recoverable by existing technology), representing 15 years reserves of crude for the USA at the 1973 consumption level.

The following chapter, on the mineralogy of oil shales, in point of fact contains a fuller account of the geology, as well as the composition, of the Green River oil shales, than that which precedes it. Succeeding chapters review retorting technology, well-logging techniques, environmental implications (of course) and the history of oil shale research over the past 30 years.

Considered overall, therefore, this book provides an important and comprehensive corpus of up-to-date information on the geology, chemistry and extractive technology of oil shale, which will be of interest to geologists, chemical engineers, government officials and other assorted doom-watchers. In common with most books from this publisher its price of about £20 (at today's rate) puts it beyond the reach of everyone except pop stars and oil sheiks. □

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Friendly persuasion on the energy crisis

John Chesshire

The Poverty of Power: Energy and the Economic Crisis. By Barry Commoner. Pp. 314. (Knopf: New York, 1976) \$10.

ALTHOUGH the world may be experiencing signs of an imminent or longer term shortage of energy, there is certainly no shortage of those prepared to write about it. This makes Barry Commoner's latest book all the more welcome because of its refreshingly different approach to energy problems. Although by training a biologist, Commoner uses the physicist's tools (the First and Second Laws of Thermodynamics and the concept of entropy) in a fluent manner to reach the apparently simple, yet powerful, conclusion that "energy is efficiently used when the quality of the source is matched to the quality demanded by the task".

The book's coverage is restricted to the USA, which accounts for over 30% of world commercial energy use, and comprises chapters on the major fossil fuels, nuclear power, solar energy sources, the energy demand sectors and the increasing capital intensity of additional conventional energy supplies.

Although the First Law of Thermodynamics has long been used to assess the efficiency of energy use, it is only since the quintupling of oil prices after October 1973 that energy studies using the Second Law have been widely undertaken. Such studies have revealed that in many instances efficiencies measured according to the precepts of the Second Law are ten times lower than the better-known First Law efficiencies. For example, the First Law efficiency of typical oil-based domestic central heating is 60%, whereas its Second Law efficiency is only about 8%. The installation of a heat-pump system, however, can raise this above 20%.

In his examination of the conflicting evidence on world fossil fuel reserves Commoner is highly sceptical of the value of estimates at present widely agreed. In the case of assessments of US crude oil reserves Commoner argues that "the declining rate of oil discovery per year is a result of company decisions to cut back on exploration efforts rather than on the

depletion of accessible oil deposits". The low economic returns from exploration in the USA have led to the increasing multinationalism of the US petroleum majors seeking lower cost (and higher profit) sources in the Middle East and elsewhere. Nevertheless, despite his optimism regarding the potential size of US and world reserves, Commoner argues that the physical fact of exhaustion, even if delayed by potentially greater resources, is reason enough to plan for the transition from the present heavy dependence on oil to renewable energy sources.

Although Commoner systematically reviews many of these benign energy sources he seems most committed in his discussion of the many issues raised by the growing utilisation of nuclear power. The Atoms for Peace program launched by Eisenhower in 1953 ushered in the widespread harnessing of nuclear power for civilian purposes, but its earlier, war-time history inevitably resulted in the nuclear power industry being a potentially explosive mixture of the civilian and military.

The economic structure of the US nuclear industry is a complex web of public and private enterprise: research and development are largely government funded; uranium mining, milling and conversion are privately funded; fuel enrichment is carried out in government-owned plants, fuel fabrication privately; commercial reactors are owned and operated by private utilities with substantial government supervision (for example, licensing and inspection), whereas all the proposed waste disposal schemes will be government controlled. In addition, since nuclear power is the only civilian technology that the insurance market is unwilling to underwrite, the Price-Anderson Act ensures substantial state settlements in the event of claims arising from a nuclear accident.

Commoner's concern with the specific technological difficulties and dangers associated with fast breeder reactors and the apparent lack of progress to date on operational long-term waste storage and disposal facilities—"a sign both of commendable caution and inadequate work"—is all the more topical given the recent Royal Commission Report on *Nuclear Power and the Environment*. Although confessing his unfamiliarity with economics, he presents a strong case based on the differential paths of increases in real capital costs and fossil fuel prices, to reveal "the likelihood that the entire nuclear program is headed for extinction". I am not totally convinced by this argument, but it is certainly the case that faced with increasingly politicised debate and

opposition to nuclear power—namely, the Swedish election and the Californian referendum—competing claims on restricted public expenditure and the energy research and development budget, rising capital costs, greater environmental safeguards, and downturns in traditionally exponential electricity growth rates, proponents of nuclear power are facing a major reassessment of its future role

In conclusion, Commoner is a persuasive commentator and despite

his strong environmentalist bias and support for solar energy, agrees that the so-called energy gap will not be "swept away in a flood of sunlight". One minor complaint: for a book which is inevitably quantitative, there are no tables or charts to ease the exposition □

John Chesshire is a Fellow of the Science Policy Research Unit, University of Sussex, UK

Energy supply and demand

Peter Chapman

Potential Energy An Analysis of World Energy Technology. By Michael Kenward. Pp. x+227 (Cambridge University. Cambridge, London and New York, September 1976) Hardcover £6.20; papercover £2.80.

ENERGY research and development is one of the few fields of inquiry that enjoys a steady growth in its level of financing, perhaps more so in the USA than in the UK. Over the past two and a half years Mike Kenward has charted the ups and downs, the fashions and flops in energy research and development in the bi-weekly 'Energy File' in *New Scientist*. He has now put together a large part of this material in *Potential Energy* which claims to be an "analysis of world energy technology". The review of energy supply options is certainly comprehensive, from solar to nuclear fusion through oil, coal, gas, fission, wind and waves. For anyone not familiar with the range of developments in these fields this book is certainly a useful starting point, but only a starting point.

There are two major defects in this book. The first is that Kenward studiously avoids trying to assess the relative merits and disadvantages of the energy technologies discussed. Occasionally we catch a glimpse of what the author's opinions are: "as far as nuclear research and development goes it is doubtful if even more spending is justified". But by and large he merely describes the technology and describes other people's reactions to the problems. This journalistic style was appropriate for a magazine feature on energy, but leaves the reader of the book in a policy vacuum. This is characterised by the platitudinous and obvious conclusion to the book which points to the twin dangers of "putting all our eggs in one basket only to discover there is a hole in the bottom"

and spreading research and development funds "so widely that no energy technology receives enough support to prove itself". Since this was, and remains, the obvious problem in formulating an energy research and development policy it seems a shame that nowhere does Kenward address himself to the problem.

A more glaring defect in Kenward's treatment of energy research and development policy is the other half of the energy question, namely energy demand. In 218 pages of text there are no more than 8 pages on factors likely to influence energy demand. This reflects the institutional blindspot to the one energy research and development area likely to pay off most handsomely in the medium term. It seems likely that with a moderate range of incentives UK energy demand could be con-

strained to increase by less than 30% over the next 25 years (compared with an increase of 60% over the past 25 years). If this potential exists with present energy utilisation technology it is likely that further research and development on energy utilisation technologies could lead to even greater savings. The known savings are cheaper by a factor of between three and ten, in both capital and total cost terms, than the equivalent increases in energy supplies. This indicates that there is considerable scope for developing additional energy-saving technologies before they cost as much as the energy supply technologies. This is recognised in some of the small print in Walter Marshall's discussion document *Energy R & D in the UK* (Department of Energy, Series of Papers on Energy, June 1976), but is ignored in practice. It seems that Kenward has been unduly influenced by the massive volume of literature and finance associated with energy supply technology and has missed perhaps the largest 'potential energy' area in an otherwise useful review. □

Peter Chapman is a Senior Lecturer in the Department of Physics, and Director of the Energy Research Group at the Open University, Milton Keynes, UK.

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Climate and landforms

Robert P. Beckinsale

Geomorphology and Climate. Edited by Edward Derbyshire. Pp. xii+512. (Wiley-Interscience: New York and London, August 1976.) £14.50; \$29.

THROUGHOUT the twentieth century the direct and indirect influence of climate on the shapes of landforms has been generally admitted, and the cult of distinctive landforms in distinctive climates has become a hallowed geographical tradition. There is much in its favour because the characteristic landforms in cold glaciated lands, hot deserts and humid tropical regions seem to differ markedly from each other. So climatic geomorphology grew up as a zonal concept wherein each broad thermal belt tended to develop its own specific complex of dynamic processes. Climate was exalted as the great panjandrum, the universal supplier of solar energy and the creator of kinetic energy for the moisture machine. Climatic geomorphologists seemed to be over-riding the doctrine of James Hutton:

"Our land has two extremities; the tops of the mountains, on the one hand, and the sea shores, on the other:—While there is a sea shore and a higher ground, there is that which is required for the system of the world: Take these away, and the world would remain an aqueous globe, in which the world would perish."

Yet it is obvious enough that before climate can work on rock surfaces, some of the latter must be raised above sealevel; once exposed they will probably consist of differing geological compositions which will withstand the attacks of weathering and erosion in different ways and at different rates. Most landforms must be considered to represent the interactions of their structure, stability relative to base level, the processes at work on them past and present, and the length of time for which those processes persisted. The complex nature of each of these parameters and the multitude of possible combinations of their variables ensure as regards surface shapes—or slopes, or whatever is meant by morphometry—that all forms of hillslope occur in all climatic environments.

The scientific analysis of the shape and spatial distribution of landforms is an enormous task still largely uncompleted yet it forms the basis of early climatic geomorphology. The later or latest trend is to determine

the exact cause and rate of the climatic process, and in turn to determine the relative importance of each major component of climate. At the same time the relative importance of the passive parameter, the rock structure, may also be revealed.

It is in this spirit that *Geomorphology and Climate* presents an analysis of the interactions between climatic processes and the shape of landforms. Written by fifteen well-known authors from Australia, Europe and North America, it can safely be said to be authoritative and certainly is neither dull nor dogmatic. It opens with a broad survey of the problems on the assumption that the central concern of climatic geomorphology lies in the extent to which variations in the elements of climate are reflected in geomorphic processes to produce distinctive suites of landforms. After a discussion of the traditional approaches by way of broad zonal patterns based largely on climate, vegetation and soils, the evidence of surface shape is shown to be inadequate as the sole basis for testing the influence of climate on landforms.

The following six chapters proceed in a logical sequence to discuss climatic interactions involved in rock weathering, mass wasting and the development of soil catenas and slopes. As would be expected of these experienced campaigners, the approach is modern, thorough and objective. For example, we learn that attempts to classify mass-movement types and frequency and their role in landscape development in terms of climatic parameters are doomed to failure; that, except in extreme situations, it is premature to relate climatic-geomorphological systems to soils; and that at medium or regional scales, slope forms cannot usefully be viewed as responses to climatic variables alone.

The next four chapters deal with water in hydrological slope models, with fluvial erosion rates, drainage networks, and, using West Malaysia as a detailed case study, with the role of climate in denudation. In these fields the influence of climate seems to be more securely established. For example, although several of the interactions are poorly understood and much more practical measurement is needed, the present findings and models of hydrological slopes are 'reasonably acceptable' and, moreover, within morpho-climatic zones the values of drainage density do reflect the climate.

Inevitably the discussion then turns, in chapters 12–14, toward lithology, which provides the framework and constraints in which climatic influences bring about the nature and rate of weathering, denudation and erosion.

There seems to be no doubt that geological factors may become dominant on a small scale and tectonic factors on a somewhat wider scale. The relative importance of rock composition is analysed with particular reference to granites, limestone, sandstones, schists and basalt. Just as the use of standard climatic means is being replaced by that of more pertinent extremes, so the use of crude rock types is being refined by the subtleties of microscopic analysis of rock composition and texture.

The concluding chapter gives a nicely rounded account of climatic factors in cirque formation. Not surprisingly, as cirques are often partly inherited from a pre-existing fluvial system and have close relationships to faults, the authors found it difficult to make generalisations on cirque variations in relation to climate.

This excursion to the snowline inadvertently draws the reader's attention to the absence of chapters on glacial, periglacial, and arid geomorphology. But the editor has already explained this omission. In his opinion, large-scale global, zonal or regional groupings, known as climatic geomorphology, reached their acme in 1950–65 and probably cannot profitably be taken much further until their broad assumptions have been fully tested by establishing the precise relationships between landform shapes and climatic parameters. Consequently this text is on geomorphology and climate.

Bear in mind, however, that in landform evolution the present dynamic processes are often not the key to the past, and that, as an American wit said, the landscape has been out of doors a long time. Because of wide swings in climatic zones during the Quaternary and the remarkable survival and reappearance of relic features from the more distant geological past, over large areas the present landforms are partly a product of past processes. But present processes do, however, provide the key to present denudation and therein lies much of the value of this collection of essays. An understanding of geomorphic processes and of their influence on the existing landscape is essential to Earth Sciences in an age when man's disturbance of the natural environment has become excessive. Consequently the advances in knowledge and experimental techniques discussed here are to be welcomed, especially as the clear exposition and frequent diagrams make them readily intelligible. □

Robert Beckinsale is an Emeritus Leverhulme Fellow and Supernumerary Fellow of University College, Oxford, UK.

Climatic change for better or worse

Hans Mörth

The Genesis Strategy: Climate and Global Survival. By Stephen H. Schneider. Pp xxi+419. (Plenum: New York and London, 1976.) \$14.95. *Forecasts, Famines and Freezes: Climate and Man's Future.* By John Gribbin. Pp. 132. (Wildwood House: London, 1976.) £4.95.

AUTHORED by one of the world's leading atmospheric research scientists who has specialised in the study of climate and its socioeconomic implications, Stephen Schneider's *The Genesis Strategy* has all the ingredients of a classic in its field. Nine chapters, ranging from strictly scientific aspects of atmospheric behaviour to the broad sociological spectrum of governmental strategy in the face of possible disaster, provide an immense wealth of food for thought. Written splendidly with the assistance of Lynn E. Mesirow, this book is likely to appeal most to those intellectuals who, despite having worked for years with logical reasoning and cold numbers, have maintained an open mind and heart for the real problems of this world. For the scientist, there is a wealth of useful references to literature on recent research in climatology and other related disciplines. Thirty-eight well selected diagrams provide the illustrations for key issues discussed in the text.

I will concentrate on the scientific atmospheric aspects of Schneider's work. A short account of recent climatic fluctuations leads up to the crucial question whether human activities have contributed to their occurrence. Inadvertent climate modification by man's activities is unquestionably gaining importance while present trends of the world's industrial activities continue. But, have we reached the point where large-scale climatic features can be produced? Although there is a general appreciation among climatologists of the basic problem (and it is a real problem), Schneider's emphasis and timing are likely to be regarded as overdone and premature. Nevertheless, in the spirit of the message conveyed by this book, there is justification for over-anxiety, as some consequences of inadvertent climate modification are irreversible once the point of no return has been passed.

One chapter is devoted to the problem "Climatic History—What if it Repeats?". Excerpts from well-documented accounts of historic climatic events provide a convenient introduction to this topic. The prehistoric 'palaeo'-climatic period, especially the occurrence of ice ages, is also briefly discussed. The lessons to be learned are manifold and important. Essentially, the study of past climates requires an interdisciplinary approach, including archaeology, geology, glaciology, history and palynology, among others.

A review of the theory of climate is presented. Here, the meteorologist reader will feel more on home ground, relatively speaking. Schneider places much emphasis on local changes of albedo on land surfaces due to differential land use, especially grazing of domestic animals. These albedo changes can be detected on photographs taken from Earth Resources Technology Satellites (ERTS). It is my opinion, however, that these are no more likely or unlikely to affect the large-scale circulation of the atmosphere than the condensation cloud trails produced by vast numbers of aircraft. Schneider does not consider the effects of the latter, whereas the bedouins are mentioned in three different contexts as possible culprits behind the Sahel drought disaster. Later in this chapter, it is conceded that climatic cause-and-effect linkages are very difficult to prove because there is still no adequate quantitative theory to explain any of the possible causes responsible for a particular climatic event.

Sixty pages of this volume deal with specific aspects of weather and climate modification. This is probably the most comprehensive presentation of topical aspects in this field available at present. In a world scarred with the effect of man's disastrous attempts to interfere with nature it is most satisfying to follow Schneider's thoughts on the advisability and wisdom of climatic control: "Control the Climatic Controllers, Not the Climate".

The climatic influence on food production, the present state and future needs of food supply for the world's population, and the dilemma which is likely to arise in the near future are portrayed with clarity and conviction in the focal chapters of this book. Following the pattern of climatic extremes which adversely affected crop production of the world's grain belts in recent years, the question of "can something be done?" looms between the lines.

Assuming that Stephen Schneider regards himself basically as an atmospheric scientist, the fundamental question arises whether scientists should pursue their professional activities

regardless or whether they should make an effort to ensure that the application of scientific knowledge to national and international problems is scientifically sound and morally justifiable. In writing this book, Schneider has emphatically affirmed his willingness to share the responsibilities towards the world. No doubt, the outspoken manner of his treatise will raise a number of eyebrows and tread on a number of toes. Perhaps, this is just what is needed in some circles of the profession which have become complacent over solving 'their' problems by finite difference steps—*ad infinitum*.

THE contents of the second book, *Climate, Famine and Freezes*, deal with two aspects: evidence for the notion that climate is changing, and scientific work dealing with this problem.

An outline of the large implications for today's economies of relatively small-scale climatic trends and fluctuations supports the view that climatic research is essential and a matter of urgency. Attention is drawn to the potentially high benefit-cost ratio for research in this field.

An account is given of recent temperature and rainfall trends, some of which have already infringed drastically on human life—for example, Sahel droughts, and Mediterranean region summer rains. Some of the statements and conclusions are fleeting and lacking in precision. The essential features of recent climatic trends, however, are well enough portrayed, and the recent drought (1975–76) in north-western Europe and the British Isles fits well into the picture.

On previous climatic changes, the author describes methods and techniques of obtaining prehistoric weather information indirectly (by proxy) from tree rings, sediment varves and pollen spectrum analysis. Attention is drawn to recent work in Africa linking equatorial rainfall with the extent of glaciation.

Implications of recent variations in the area covered by ice and snow in the Northern Hemisphere on the weather and climate, are discussed. Any hints at the likely causes of these changes must at this stage be regarded as pure speculation.

Gribbin devotes a chapter to the question of solar influences on weather—a most controversial and debated aspect of atmospheric science. Whatever the reader's view on this matter he must concede that the information given makes interesting reading.

Climatic trends of a long-term nature and their derivation from historical and prehistoric evidence are discussed. This work is gaining importance with regard to climatic forecasting as it delineates the boundaries within which the climate varied in the past. As one climatologist

put it: "What happened in the past can happen again". Unfortunately this theme leads Gribbin to speculate on solar-terrestrial weather relationships of a kind for which no scientific proof will be possible in the foreseeable future.

One of the most recently explored geophysical aspects of weather and climate is the Earth's magnetic field. Although this subject may be anathema to those meteorologists who believe that one can well manage without it, there is certainly justification for research, especially as good long-term records exist. At this stage of affairs, one simply cannot afford to reject outright a possible influence on, or association with, climate and its variations.

A better understood, but equally difficult, field of meteorology is the penetration of solar radiation through the atmosphere; or, looking at the other side of the coin, the loss of surface heating through interception of solar radiation by dust, clouds and aerosol in the atmosphere. The author correctly stresses the sensitivity of polar and sub-polar ice and snow cover to the delicate heat balance in high latitudes.

The question whether man's activities are changing the climate is dealt with in its widest terms. The author's treatment reflects the present majority opinion among scientists working in this field: although evidence for small-scale effects already exists (urban heat islands, rainfall modification, and so on), the large-scale atmospheric circulation is still overwhelmingly governed by "natural" events.

An objective and balanced view is taken in the discussion of the prospect for an early and sudden reversal to ice-age conditions. Major theories of global cooling are presented and put into perspective. One passage in the description of the astronomical theory of ice ages—"The most extreme effect by this process reduces the intensity of sunshine reaching the Earth by 30%"—is likely to produce an erroneous impression in the reader. Incidentally, Gribbin is in very good company when tackling this explanation, as Sir John Herschel may also be accused of misleading the readers of his *Outlines of Astronomy* (1875 edition) when he wrote: "... by concentrating half the annual supply into a summer of very short duration and the other half over a long and dreary winter, . . .". When Gribbin writes that the world's climate involves more complex factors than a simple dependence on variations in solar energy produced by changes in the Earth's orbital motion, he really puts the finger on the right spot.

Another important point is brought out in the discussion of the limitations to conventional forecasting techniques. Numerical computerised forecasting

techniques of the kind applied at present to the production of short-term (up to 72 h) forecasts cannot be stretched to cover longer periods while still providing acceptable results. Development of long-term forecasting requires the inputs of different kinds and timescales, including slow-acting feedback mechanisms, such as may be produced by changes in ocean surface temperatures. Present attempts at climatic forecasting are very much in the experimental stage.

Evidence for the association of grain and food production fluctuations with the sunspot cycle is scanty and covers too short a period. Surely a good number of the ups and downs of agriculture around the world are bound to coincide with sunspot maxima and minima. What about the remainder? More relevant and indeed important is the problem of crop failures due to weather extremes in our times when the world's

food reserves are nearly exhausted. Gribbin draws a far-reaching conclusion: that the economic policies of many countries may have to be altered.

In a postscript, the ultra-long aspect of changes in the Sun's radiation due to the Solar System passing through dust lanes in the spiral galaxy are considered. Gribbin's flare for an astronomical view on the problem is appreciated.

This book is a well written popular account of the state of the art of explaining the past and speculating on future climatic changes. It contains the essentials of present knowledge of this subject. Good bed-time reading for the layman and unbiased meteorologist. □

Hans Mörth is Deputy Director of the Climatic Research Unit, School of Environmental Sciences, University of East Anglia, UK.

Mathematical models in ecology

John H. Lawton

Theoretical Ecology: Principles and Applications. Edited by R. M. May. Pp. viii+317. (Blackwell Scientific: Oxford, London, Edinburgh and Melbourne, 1976.) Cloth £8.50; paper £4.80.

I ONCE heard Charles Elton say that the trouble with ecology was that it was "fun to do, but not very interesting to read about". This book makes it abundantly clear that ecology is now not only exciting to do, but also makes excellent reading.

The aims of the book are very simple: it seeks to review and draw together a whole series of mathematical models, to show how they can shed light on empirical observations and to examine some of the practical implications. In so doing, it occupies a niche between the numerous large general textbooks of ecology that have appeared recently, and the more technical literature that cascades on the world in the form of journals and specialist monographs. It embraces a range of subjects reflecting the core of current interest in theoretical ecology.

Certain relatively small areas receive scant or no mention (for example, optimal foraging theory, or spatial patterning in vegetation), and the book draws almost entirely on theoretical models that can be handled analytically. Large scale computer simulations

('dirty great' as opposed to 'little dirty') models figure hardly at all. It is also a theoretical edifice built on an inverted pyramid of biomass, with animal examples well in excess of plant examples. These are not criticisms.

The organisation of what the book does cover is conventional, and sensible. It starts with an examination of the dynamics of single species populations by May, and is followed by a review of the evolution of major population characteristics by Southwood. Between them, they illustrate particularly well something of the how and why a population's characteristics are as they are (in the resources allocated to reproduction, longevity, and so on), and how these characteristics influence its dynamics. Interactions between two populations are considered in the next four chapters, which deal first (by May) with some of the general features of predation, competition and, less conventionally, mutualism; and then in more detail with arthropod predator-prey interactions (by Hassell), plant-herbivore systems (by Caughley) and competition and niche theory (by Pianka). One of the more interesting things about ecology is the possibility of advance from a consideration of the dynamics of a single species or pairs of species and ignoring all the rest. It looks like cheating, but it also seems to work, as the interplay between theory and practice developed in these first six chapters clearly illustrates. In reality, perhaps many ecological systems are very loosely connected in terms of the major controlling links between species.

In certain cases, of course, it is misleading or nonsense to confine attention to single species, or pair-wise interactions. Various multi-species problems

are therefore considered in the next three chapters, which deal respectively with patterns in multi-species communities (by May), island biogeography and the design of nature reserves (by Diamond and May), and succession (by Horn).

The remaining four chapters would not normally be classified in the mainstream of theoretical ecology—friends or half-cousins perhaps, but not close family. In fact, they link rather well with the rest of the book, and serve to remind us that the traditional boundaries of the subject are in a state of flux. Wilson provides a brief overview of some of the central problems of sociobiology as he sees them and forces the reader to think about such problems as group selection, the evolution of sex, and adaptive demography. Gould expands the timescale of traditional ecological thought and attempts to bridge the ravine between palaeontology and ecology; and Cohen directs our thoughts inwards to the biological and ecological puzzles presented by our schistosome parasites. Finally Conway shows how ecological ideas can provide important insights into the behaviour of another group of nasty animals—insect pests.

Two criticisms are frequently levelled at multi-author texts: lack of cohesion and a large variance in performance. Neither can fairly be levelled at this book. Each author takes particular pains to draw on, and refer to other parts of the book. To give but two examples. Conway makes very successful use of the concept of the 'r-K' spectrum documented at length by Southwood, and Gould is able to use the species-area relationships that form the backbone of the chapter by Diamond and May, to provide a convincing explanation for one of palaeontology's biggest paradoxes—the Permian crisis of extinction. This interplay of ideas is obvious throughout the book. The papers do vary in quality, of course; all are worth reading, and most are of the highest standard. I found the paper by Caughley the most disappointing, in that it makes the least convincing contact of all between the real world and the models, but I suspect this is as much a fault of the lack of data as anything else.

This book will surely be essential reading for all serious students of the subject (both advanced undergraduates and postgraduates), and a valuable source of authoritative summaries and guide to the literature for teachers and research workers, for several years ahead. Not only will they learn a lot: they ought to enjoy it. I did. □

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Room with a view

K. G. Eldridge

The Natural History of Trees. (The World Naturalist Series.) By Herbert L. Edlin. Pp. xvi+269+66 plates. (Weidenfeld and Nicolson: London, September 1976.) £10.

HERBERT EDLIN looks out at trees and forests of the world from the window of a country house not far from London. There is much to be seen and learned nearby, and much more to be remembered from his earlier experiences in other lands.

There are fine precedents for his approach to writing a natural history book: *The Origin of Species* was written from a house with a similar view. Edlin's latest book, however, does not present any grand concept or develop a thesis. It is descriptive, aiming to present factual information which might form the basis of ideas and rational action.

Administrators, politicians, some scientists and engineers, students, and other people who argue about the use of forest land, whether to chop trees down or grow more, often talk in ignorance of the basic biology of trees. This book will be a useful introduction to informed discussion.

Edlin deplores policies of single use of forests and advocates prudent multiple use. He does not like the current single-use ideas of wilderness, conservation, restricting access and allowing little or no management. Nevertheless he agrees that forest conservationists have reason to be active and angry. He shares their outrage at the single-use policy called "cut out and get out" which has exhausted much of the conifer forests of the west coast of North America.

People do not put a high value on their forests while wood and other products are available in unlimited amounts at no cost, like fresh air. The value of these commodities only becomes apparent as they are depleted or obliterated. With forests valued more highly and with a greater understanding of forest biology Edlin expects some of the dilemmas of the use of forest land to be resolved.

As a naturalist he has an obvious pleasure in trees and in understanding the natural processes of their life. His descriptive writing is attractive and clear, touching on every aspect of the

biology of trees both as individuals and in communities. Following the familiar pattern of the older textbooks of forest botany he describes the anatomy and development of seeds and seedlings, wood and trees, the ascent of sap, and the annual cycles of leaves and flowers. The descriptions are brief and accurate but the illustrations are inadequate. Good illustrations are essential to supplement the description of the cambium and how it makes wood, or of the guard cells of stomata and the way they let water vapour and air in and out of leaves.

Throughout the book Edlin shows a preference for writing of "purpose" rather than of "function", of "winged seeds designed for wind dispersal", as though the tree had decided and acted on what was best for itself. Such a teleological explanation seems simple, as it avoids the issue of evolution. Unfortunately it denies the reader the great satisfaction, which Julian Huxley and other splendid English writers on natural history gave us, of recognising adaptations as being the result of natural selection.

The descriptions of trees in communities in relation to soil, light and climate show how one kind of tree replaces another and the dependence of trees on their environment. The crowns of large trees in a forest are exposed to the macroclimate of the district, while the floor of the forest has a calmer, darker, more humid environment with less fluctuation in temperature. The author shows a special interest in the effects of wind on trees.

His outlines on forest soils and soil erosion give little hint of the vast amount of information on those subjects. In fact he could have mentioned several textbooks for every page of the whole book, but there are no references to any other publications.

With the scientific forester's approach of "wise management for sustained yield" he describes the many sorts of forests throughout the world and the ways they are used. In his account of the characteristics of broad-leaved and conifer forests of the Northern Hemisphere his explanations of the distribution of species are too brief to be satisfactory. One might agree at first that conifers predominate towards the Arctic because it is an advantage for a tree to be evergreen and ready to grow immediately the temperature is warmer than 6 °C. Edlin does not, however, discuss the rapid growth of the deciduous birch which is always present with the pine and spruce at the far northern timberline.

There is a similar lack of space to do justice to the complex ecology of the open savanna forests of tropical countries. Small trees grow in a grass-

land sustained by man-made fires, but when eucalypts are planted they soon outstrip the native trees. A recent explanation, which the author does not mention, is that the roots of foreign trees can penetrate a hard layer deep in the soil, which is impervious to the roots of native trees, and obtain water from greater depths in the dry season.

The material in the book is more about the trees and forests of southern England than elsewhere. Consequently its greatest appeal is likely to be to those readers close to the writer's familiar home ground who also would like a glance at living forests of the rest of the world.

Some of the most attractive parts of the book are those in which the writer gives an account from his personal experience of a forester's daily life in England, of a Malaysian rubber plantation and of rainforests. Edlin gives a lively description of the complex, diverse and changing tropical rainforest and notes with regret that the modern rainforest "is more likely to be raped than virgin".

Being an experienced writer in his field of popular forest science, Edlin

is concerned to be correct in his factual information. The few minor inaccuracies I noticed probably resulted from the brevity needed to fit such a large number of topics into 269 pages. For example, it is not correct to say the natural range of *Eucalyptus saligna* is in a Mediterranean climate. It is in an area of predominantly summer rainfall grading into a winter rainfall, Mediterranean climate at the southern end. Also, it is not correct to suggest that sulphite pulps are the main raw material for small paper bags, although they are sometimes used.

It was a curious decision in an otherwise well-produced reference book to have no suggestions for further reading to encourage the interest aroused in students. There should have been room for a bibliography and also a glossary. Nevertheless Edlin's book is readable and informative and will be a useful general reference for newcomers to the study of trees. □

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Broad canvas for cytoplasmic genetics

R. A. E. Tilney-Bassett

Cytoplasmic Genetics and Evolution. By Paul Grun. Pp. xi+435. (Columbia University: New York, July 1976.) \$31.25.

THE publication of a book in the field of extranuclear inheritance is a rare and welcome event. Paul Grun begins by establishing the physical properties of mitochondria and plastids, and their potential for genetic variation through mutation, segregation and recombination. A few of the more important mitochondrial and plastid enzymes are each briefly described, as if the author wished to emphasise that he had not completely neglected the biosynthetic capabilities. But, in my view, this was insufficient to serve much purpose and a greater attention towards clarifying the often quite complicated genetical accounts would have been more valuable. Nevertheless, I was impressed by the way in which the reader is briefly and carefully guided through the experimental procedures, and by the way the evidence is sifted and both sides of controversial issues discussed.

A large section on symbiotes and inherited diseases extends cytoplasmic inheritance into consideration of the relationships between bacteria and their insect hosts, spirochetes in *Drosophila*, killer paramecia and killer yeasts, and the problem of cancers in vertebrate animals. Non-cancerous diseases are also discussed using the example of scrapie in sheep and Leber's optic neuritis in man to illustrate the importance of understanding the interactions between nuclear genes and transmissible factors. Yet, surprisingly, Grun fails to drive home the message that probably several other diseases might be better understood if regarded in this light. Having opened the door to discuss symbiotes, I was disappointed to find no mention of those remarkable chloroplasts that can exist endosymbiotically in the cells of some molluscs and other invertebrates.

The final of the four parts catalogues many examples illustrating the importance of the plasmon as the causative factor in determining some of the stranger facts of cytoplasmic genetics. Particular attention is given to the interaction between nuclear genes and plasmon factors, as for example in cytoplasmic male sterility, and to the evidence for genetic variability and evolutionary change within the plasmon. The chapters of this part add up to an extensive and thorough review reflecting the author's close familiarity with this little understood subject—a genetic backwater that needs this kind of publicity.

To bring together into one book such a wide range of genetic phenomena is a Herculean feat—over one thousand references are quoted. But Paul Grun is not content with pure description; he has attempted a modern synthesis in which he relates cytoplasmic phenomena to varying states of an overall evolutionary process. Whether we look at a parasite starting to invade a new host, or a long established organelle like a mitochondrion, there is a continuing process of genetic and physiological adjustment by which host and invader work out a strategy to their mutual advantage. Gradually, the successful parasite may become the universal symbiont and essential organelle. Initially the host may not be able to resist invasion, yet slowly and inexorably, through mutation and selection, the host nucleus gains control. At this stage any major disturbance to the harmonious relationship between the two is strongly selected against, and so the interaction between nucleus and cytoplasm becomes a restrictive force on evolutionary divergence. So close and so delicate is the interaction, that we find many examples of geographical races or neighbouring species, in both plants and animals, that fail to form healthy fertile hybrids because the hybrid nucleus is incompatible with the maternally inherited cytoplasm. In short, the cytoplasm has become an important force in evolution.

The evolution of a mechanism to limit the transmission of cytoplasmic factors to the female line is seen as a strategy by which the host effectively stops the recombination of cytoplasmic genes and thus gains mastery over their behaviour. Once sufficient control is acquired, the host nucleus may even take over some organelle genes as seems to have happened in the splitting of the cistrons for certain plastid enzymes between nuclear and plastid DNA, and in the reduction in coding capacity of vertebrate mitochondria compared with that of yeast or higher plants.

Although some areas are covered too thinly, the author's aim to paint a broad canvas covering the more important aspects of cytoplasmic genetics is generally successful. Above all, the illustrated book is written in an easy style, and it makes such fascinating and enjoyable reading that it should have a wide appeal to degree students in the biological sciences as well as to research workers. It is to be hoped that the high cost does not prove too great a discouragement for a book that deserves to be widely read. □

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Modern microbiology

Sydney Selwyn

The Development of Microbiology. By Patrick Collard. Pp. 212+xx photos. (Cambridge University: Cambridge, London and New York, November 1976.) £6.50.

THERE is probably no field of science or medicine which has a more fascinating and exciting history than microbiology. Paul de Kruif's classic, if over-romanticised, epic *The Microbe Hunters* has undoubtedly stimulated many young people to enter the medical and related professions during the 50 years since it was first published. The same source provided initial inspiration for the recent British television (BBC2) series *Microbes and Men*. The enthusiastic reception which greeted these dramatised sequences showed that the history of medical microbiology maintains a very wide appeal.

Paradoxically, however, this enthralling subject has received relatively little serious historical study. William Bulloch's *History of Bacteriology* stands alone as a detailed but idiosyncratic work. First published in 1938 it is now, sadly, unavailable. A briefer account by W. W. Ford, written in 1939, was followed more recently by W. D. Foster's general history. This contains a good survey of important French and German achievements of the nineteenth century but it neglects the earlier (and most recent) periods, and much of the less familiar British work. A very welcome event, therefore, is the publication of a new historical survey by Professor Collard of the Manchester Medical School. It arose from lectures to postgraduate students of medical microbiology and is admirably suited for such an audience. The cognoscenti, too, will find many delights but these will inevitably be tempered by disappointments especially in relation to the 'archeological' period before Pasteur.

The book is divided in a very practical way into thirteen main chapters which consider in turn the development of such aspects as microscopy, microbial cultivation, chemotherapy, genetics and serology. The author has wisely resisted the temptation to deal chronologically with the entire subject *en masse*. At the outset he delineates four eras through which microbiology has passed: speculation, from 5000 BC

to 1675; observation, from 1675 to the mid-nineteenth century; cultivation, to the beginning of the twentieth century; and the era of physiological study, persisting to the present day.

Although this view has the merit of simplicity, it presents a static picture of a dynamic subject. In particular, it ignores the fact that the volume and quantity of speculative writing actually continued to rise steadily after 1675. The later landmarks include Benjamin Marten's book on consumption of 1720 and Richard Bradley's treatise on the plague of the following year, but hundreds of others could be cited, culminating, during the mid-nineteenth century, in the writings of Jacob Henle on infection in general, William Budd on typhoid and John Snow on cholera.

Perhaps surprisingly, the greater part of this work was British—as was a remarkable amount of more practical investigation. Microscopy begins logically with the elegant treatises on lenses by Roger Bacon and other Oxford philosophers of the thirteenth century, proceeding through the work of such forgotten figures as Leonard Digges, the elder, in the mid-sixteenth century.

The first practical microbiologist, however, was probably Thomas Moffett. This English physician, who is likely to have been the father of the celebrated "little Miss Muffet", not only described the infective cycle of silkworm disease, but also in 1589 provided a practical basis for a germ theory of disease with his study of the biology, pathology and treatment of scabies. Although Moffett is the undoubted founder of parasitology—and conceptually, of medical microbiology—he is totally neglected by the historians of these subjects. So also is one of the greatest theoreticians of all time, Francis Bacon, whose writings contain the roots of all that was to follow in microbiology. His assertion that an understanding of the processes underlying fermentation and putrefaction would be the key to the mysteries of disease and other "operations of nature" forms the basis for the later researches of Boyle, Willis, Pringle and their successors. Similarly, his perceptive remarks on the potential value of the new microscope lead directly to the work of that remarkable group of pioneer microscopists of the mid-seventeenth century—Mundy, Charleton, Power, Grew and Hooke. Only the latter has received any attention by historians of microbiology, and Professor Collard gives him merely passing mention.

Van Leeuwenhoek receives a more adequate amount of space, but some misconceptions need to be dispelled. He did not arise *deus ex machina* but

his single-lens microscopes are perfect replicas of the type fully described ten years earlier by Robert Hooke, who also provided the idea of modified dark-ground illumination which probably determined van Leeuwenhoek's later successes. The Dutch draper was, moreover, incapable of producing the drawings contained in his famous letters to the Royal Society. Clifford Dobell, his reverential biographer, describes the mediocre quality of the work done for van Leeuwenhoek by his draughtsmen. So much so, that Professor Collard identifies "streptococci" from a sketch intended to portray, with a series of dots, the track of a mobile bacillus.

There are few such problems of interpretation in the modern period, although the story of Fleming's rediscovery of penicillin needs overhauling following Ronald Hare's revelations. Also Domagk's prontosil red was virtually inactive *in vitro* and was relatively ineffective against pneumococci *in vivo*. These and other minor errors should not be allowed to dissipate our genuine gratitude to the author. He has written the best available account of the development of modern microbiology. □

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Imaginative processes come of age

P. W. Hawkes

Optical Information Processing. Edited by Yu. E. Nesterikhin, G. W. Stroke and W. E. Kock. Pp. xiv+401. (Plenum: New York and London, 1976.) \$39. *Digital Picture Processing.* By A. Rosenfeld and A. C. Kak. Pp. xii+457. (Academic: New York and London, 1976.) \$28.50. *Picture Processing and Digital Filtering.* Edited by T. S. Huang. Pp. xiii+289. (Springer: Berlin and New York, 1975.) DM79.80; \$34.40. *Principles of Optics.* By M. Born and E. Wolf. Pp. xxviii+808. (Pergamon: Oxford and New York, 1975.) £10.50; \$22.50.

TEN years ago, digital image processing hardly existed and optical processing was in its infancy. Microscopists, radiographers, archaeologists studying aerial photographs and a host of other users of images of some kind were well aware of the shortcomings of their interpretative techniques and they at best overcame or at worst disguised them by slowly accumulated experience. Nevertheless, optical processing cannot truly be described as a new subject, for it consists in combining a thorough knowledge of the optics of the image-forming process with some ingenious optical designing intended to avoid the most troublesome of the inherent limitations, probably at the expense of worsening less harmful ones: apodisation is an obvious example. The sudden interest in optical processing in the 1960s was stimulated by the arrival of the laser, which permitted essentially coherent processing, although complementary incoherent techniques have also emerged or been reconsidered in the general revival of studies in this domain.

Digital image processing is, on the other hand, a genuinely new subject, which could not exist before fast computers with very large memories became widely disseminated. To a computer, an image is no more than a two-dimensional array, usually as large as possible, of real or complex quantities; and the challenge of computer processing is a complex one: the discrete array must represent the recorded image, however degraded this may have been in the image-forming process, as faithfully as possible. This means studying the properties of the photographic medium and the optical properties of

the microdensitometer (unless the image is transmitted sequentially, as in a scanning device for example). Optimum strategies must be devised for rendering the discrete image better, in some carefully defined sense, or more informative, in the sense that the eye can recognise detail of interest more easily or that a quantitative measure is obtained of some property about which only qualitative information had been available. Finally, the processed image must be made visible preferably in a way that makes obvious its superiority over the original.

A number of books and review articles on coherent optical processing, many devoted mainly to holography, have appeared over the past few years and the volume by Nesterikhin *et al.*, which contains the seventeen papers delivered at a US-USSR joint seminar in 1975, is an uneven addition to this body of literature. In particular, some authors have been at pains to situate their work relative to earlier contributions, whereas others merely give a bald account of their own research; since there seems to have been no length limit—one chapter, an excellent one on the information content of diffraction patterns by B. J. Thompson, is 34 typed pages long—this approach is difficult to forgive. There are chapters on both the technology and the theory of optical processing, ranging from one on materials and devices by Casasent and another on holographic memories by Vander Lugt to a survey of speckle by Goodman and a discussion of holographic memory devices for specific integral transforms by Gibin and Tverdokhle. It is useful to see the lines which Russian scientists are following, as the only other sources of Eastern material, original articles apart, are the occasional Sborniki that have been compiled. Finally, a word about the editing: the book has been offset directly from typewritten texts submitted by the authors. For the price, the editors could surely have had the text retyped uniformly which would not only have produced a less scrappy-looking text but also have permitted genuine editing: the removal of such bizarre transliterations as "the Fuco-Hilbert transform" for example and the provision of some assistance to authors whose English was shaky.

In the field of digital processing, a torrent of books, many of a very high standard, has recently flooded on to the market, supplementing and replacing the excellent early texts by Andrews and Rosenfeld (by 'early' in this context we mean 1970 and 1969, respectively); indeed, the volume by Rosenfeld and Kak supercedes Rosenfeld's earlier book, and a new and expanded edition of Andrews' text is promised for the new year. The volume

by Rosenfeld and Kak and that edited by Huang contain essentially similar material, concerned with the mathematical representation of images in discrete form, compression of the information, often highly redundant, contained in them, the enhancement of contrast and the restoration of degraded pictures and some pattern recognition techniques; the latter are, however, much more fully described in other recent books not noticed here.

Picture Processing and Digital Filtering contains six contributions: a clear survey by Huang, putting the detailed chapters in perspective; an extremely good account by Andrews of most of the two-dimensional transforms in algebraic terms; separate chapters on recursive and non-recursive filters by Read, Shanks and Treitel, and by Fiasconaro, respectively; by far the best available account of mathematical techniques for image enhancement and restoration by Frieden; and a good account of the effect of noise, firmly related to the type of equipment used at present in image processing, by Billingsley. All these chapters are extremely clearly written and above all, given the novel status of the subject, they are written so that the techniques can be used and developed for a wide range of possible applications. The only disappointing contribution is that on recursive filtering, as yet a rather shadowy aspect of image processing, in which the authors never really explain what their techniques are for. But we should probably be grateful for any extended essay on this topic, whatever its shortcomings. The chapters by Andrews and Frieden are masterly and would alone make the book highly desirable.

The book by Rosenfeld and Kak is also very good indeed; although a direct competitor, in the sense that it has a great deal of material in common with Huang's volume, the tone and approach are subtly different—due no doubt to Rosenfeld's preoccupation with pattern recognition and picture descriptors during the past few years. The homogeneity of the writing is pleasant after the inevitable unevenness of the multi-author volume, and Rosenfeld and Kak have devoted a great deal of space and effort to ensuring that the reader grasps the purpose of the many techniques described. Furthermore, whereas Huang's book has the merit that a great many techniques are described by their originators, Rosenfeld's has the complementary merit of comparing and contrasting the same techniques from another viewpoint. In conclusion, most workers in this field will want to have access at least to both of these volumes; newcomers are probably better served by Rosenfeld and Kak's *Digital Picture Processing*.

Finally, we turn to *Principles of Optics*; it is always a pleasure to praise this book, now in its fifth edition. Most of the earlier 'editions' were scarcely more than reprints, as was made clear in the prefaces. But in the fifth edition, much of the material on the optical properties of metals has been rewritten, where the original text was misleading. Although sympathising entirely with Professor Wolf's reluctance to tamper with the text piecemeal in an attempt to bring it all up to date, it would be particularly pleasing if he consented to incorporate into the sixth edition the new material recently published by his own group in the field of partial coherence and the radiometric quantities. Even so, it remains a near-definitive account of classical optics at a very reasonable price. It is included in this group of books on image processing since a thorough grasp of the chapters on image formation at least is indispensable for anyone seriously embarking on digital or optical processing.

How far has image processing got

in practice? In some respects, the mathematics has outstripped the practical applications with the result that the same test images tend to recur repeatedly after undergoing a wide variety of treatments. Nevertheless, the volumes on digital methods may leave the misleading impression that theory and practice are at present far apart, for there is little mention of specific applications: X-ray tomography, for example, or three-dimensional reconstruction of electron micrographs. Despite this, the possibilities are very exciting: solution of the phase problem; extrapolation beyond the classical limit of resolution; the acceleration of picture processing using fast transforms other than the FFT; and many others. The second decade will undoubtedly witness as many innovations as the first, no less unpredictable. □

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Proprioception

J. W. S. Pringle

Structure and Function of Proprioceptors in the Invertebrates. Edited by P. J. Mill. Pp. xviii+686. (Chapman and Hall: London; Distributed in USA by Halsted Press; August 1976.) £19.50.

A BIOLOGIST considering whether to read (or purchase) a volume with multiple authorship does well to consider not only the field covered but also the standing of the contributors. The editor's tasks is like that of a building contractor: he cannot construct well without good materials. Of this book it can be said at the start that the authors are well chosen. They come from Australia, France, Germany and the USA as well as from the UK, and have all developed their insight into the subject by original contributions to it.

In 1938, I published what may have been the first paper on Proprioception in Arthropods. Now, 38 years later, 419 out of the volume's 686 pages deal exclusively with this Phylum, and as much is known about them as about vertebrate animals. The diversity of form and function is much greater. A definitive textbook on vertebrate proprioceptors has been written by one man; for invertebrates the task requires a contributory volume and an editor like this one, prepared to identify the most qualified contributors and to perform such integration as is possible.

The chosen definition of 'proprioceptors' is not Sherrington's—his task was easier because he was considering only the vertebrates; this volume covers all "sense organs capable of registering continuously deformation and stresses in the body due to the animal's own movement or to its weight or to other external mechanical forces". So defined, the vertebrate semicircular canals, otoliths and lateral line would be proprioceptors, which may seem absurd. But such a wide definition is indeed necessary for invertebrates, and if this volume is nearly a textbook of invertebrate mechanoreception it is no less valuable as that.

Laverack's Chapter 1 is largely a structural review and well illustrates the diversity. In Chapter 2, Fields, in a review of all aspects of the physiology of the crustacean muscle receptor organ, includes a clear statement of the evidence for a causal relationship between the electrogenic sodium pump and adaptation of impulse frequency, the latest demonstration of the value of this preparation for basic sensory physiology. Our knowledge of the mode of action and behavioural utilisation of this organ is at least as complete as for the vertebrate muscle spindle. Bush follows this with an account of another preparation important to general physiology—the only sense organ known to operate without impulses.

In Chapter 4, Finlayson gives us a comprehensive review of the multiterminal neurone system of arthropods, the importance of which has only recently been fully appreciated. It seems to be responsible for the overall co-

ordination of movement necessary in a primitively soft-bodied multisegmental animal and to provide the main feedbacks by which the intrinsic motor patterns are adjusted to a variable environment. But neither in locomotion nor in the specialised mouthpart and gut receptors (chapter 5 by Wales) is it possible yet to decide whether these multiterminal type II receptors and the chordotonal type I receptors (chapter 6 by Mill) serve a distinct or a complementary function in proprioception.

At the base of the decapod leg are two peculiar groups of internal chordotonal organs which neither cross a limb joint nor are attached to a muscle or apodeme. In chapter 7, Clarac considers the probable role of these organs in the autotomy reflex. The arthropod section of the volume ends with a long chapter by Wright on limb and wing receptors in insects, chelicerates and myriapods, a detailed description by Moulins of the ultrastructure of chordotonal organs and a valuable chapter by Macmillan on apodeme tension receptors. The diagram which this last author gives comparing vertebrate and arthropod proprioceptive systems will no doubt be much used by lecturers, but ought to be viewed with caution in the present state of our knowledge.

As Dorsett points out at the beginning of chapter 11, proprioception in many soft-bodied invertebrates is not the well-differentiated system found in vertebrates and arthropods possessing a rigid skeleton. Most of the locomotor activity of these animals is stereotyped and is pre-programmed in the central nervous system. Orientation to gravity by means of statocysts is, however, widespread and is included under the definition used for the volume; but proprioceptors *sensu stricto* have only been established in a few cases. Spatial equilibrium in arthropods (chapter 12 by Sandeman) needs separate treatment since the necessary information may be obtained, without a special sense organ, by monitoring the relative position of the parts of the body under linear and angular accelerations. Molluscs (chapter 13 by Budelmann) usually have statocysts.

The final chapters of the volume deal with the processing of proprioceptive information. Wells considers its role in learning; Mill and Price give the necessary theory of feedbacks; and Wiersman writes generally on various relevant topics. The editor's final brief contribution picks up some loose ends and highlights residual problems.

Altogether a most useful book, well produced and not unduly expensive. □

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American response to Darwin

David L. Hull

Darwin in America: The Intellectual Response, 1865-1912. By Cynthia Eagle Russett. Pp. ix+228. (Freeman: San Francisco and Reading, May 1976.) Cloth £6.30; paper £3.20.

RUSSETT'S well-balanced account of the intellectual response to Darwinism in America further enhances the richly illustrated picture which we already have of the Darwinian revolution. As Russett would be the first to agree, her book could have been entitled just as readily *Spencer in America* because the authors which Russett treats were as likely to have their minds blown to bits by reading Spencer's *First Principles* (1862) as by Darwin's *Origin of Species* (1859). The genuinely scientific character of Darwin's theory legiti-

mised Spencer's Cosmic Philosophy, but the actual content of Darwin's theory had as little impact on its converts as on its opponents.

Russett deals exhaustively with all aspects of 'Darwinism' in America—scientific, philosophical, religious, social, historiographic, economic and literary. In spite of the opposition of the most powerful scientist in the United States, Louis Agassiz, and perversely to some extent because of it, evolution was almost universally accepted by American biologists within a decade after publication of the *Origin*, but the 'evolutionism' which became so popular tended to be Lamarckian and teleological—partly for scientific reasons, partly theological. Theologians were not alone in viewing the death of teleology as the death of God. Yet in slaying Paley, Darwin slew a corpse. Darwin merely shocked nineteenth-century intellectuals into realising how vacuous teleology had become. The Metaphysical Club at Cambridge was the focus of evolutionism among American philosophers. As might be expected, John Fiske, the most enthusiastic evolutionist, was a Spencerian. More serious philosophers such as William James, John Dewey, Josiah Royce, and Chauncey Wright, however, viewed Spencer as so much rubbish which had to be cleared from their path before proceeding on their way. Spencer was the "philosopher whom those who have no other philosopher appreciate" (p17).

The most interesting sections in Russett's book are those in which she deals with Social Darwinism and its effects on such novelists as Jack London, Frank Norris and Theodore Dreiser. Rugged individualism, socialism, aid to the poor, free will and determinism, nature-nurture, the relative superiority of the races, group selection and altruism—it is all here but presented more bluntly and at a safer distance than it is in the current brouhaha over sociobiology. Darwin also legitimised the 'historical method', which for Dewey was the discovery of the particular sequence of conditions which brought about a particular natural phenomenon, for Henry Adams the search for the one great law of history. Adams found his great law in a combination of thermodynamics and the law of inverse squares. Not only was the universe running down, it was doing so logarithmically. The religious phase of human history had lasted 90,000 yr, the mechanical phase would last 300 yr, the electrical 17.5 yr, and the ethereal phase but 4 yr.

Russett's prevailing concern is the interconnections between intellectual disciplines. For example, if Darwin and Wallace actually borrowed the notion



Charles Darwin

Photo: Mansell Collection, London, UK

of natural selection from Malthus's *Essay on Population* (1798), then the alacrity with which economists like Thorstein Veblen read the basic principles of evolutionary theory back into human affairs was understandable. A quick glance at Malthus's *Essay*, however, brings the argument full circle because in it Malthus justifies his views about human population by reference to biological species in a state of nature. As complex as the interconnections between intellectual disciplines may be, Russett believes that the content of scientific theories can colour the general intellectual climate and *vice versa*. Her final chapter concerns the blow which Maxwell's equations, quantum theory and relativity theory dealt to the absolute, certain, deterministic, mechanistic worldview initiated by Newton.

The book is illustrated with the usual portraits as well as with extremely well-chosen cartoons and paintings. Some of these illustrations convey messages much more powerfully than any printed word could hope to do; for example, the contrast between 'Nature red in tooth and claw' and the cloying romanticism of Albert Bierstadt's painting of the Rocky Mountains. My only criticism is that Russett completely ignores the really excellent scholarship which has been produced during the past dozen years or so on Darwin and Darwinism. If she has read any of the more recent work, she does not mention it. □

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review article

Products of the major histocompatibility complex and their relationship to the immune response

Alan Munro & Susan Bright*

The genes of the major histocompatibility complex were first known for the part they played in transplant rejection. Recently, however, it has become clear that the products of that region have an important part to play in the control of the immune response, through their effects both on cooperative and on aggressive interactions between cells. It is now possible to guess at the mechanisms which may underly the association of some major histocompatibility antigens with disease.

IN mammals there seems to be a single genetic region which has a major influence on graft rejection and is known as the major histocompatibility complex (MHC). In recent years there has been increasing interest in the products of this genetic region because of their importance not only in transplantation but in many other aspects of immunology¹⁻³.

The MHC is divided into different regions mainly on the basis of genetic recombinants⁴. The probable arrangement of these regions in mouse and man are shown in Fig. 1. In this review we want to discuss the properties of the MHC as a whole, in particular its unusual genetic properties and the association of certain alleles in the MHC with diseases in man. In order to do this it is essential first to discuss the properties of the genes which lie in the different regions of the MHC, in terms of their genetic properties and in terms of the molecular nature and suggested functions of their products. In this connection it is helpful to compare and contrast the properties of corresponding genes found in mouse and man. We shall then return to the problem of disease association with HLA in man and finally discuss the possible reasons for the unusual genetic properties of the region as a whole.

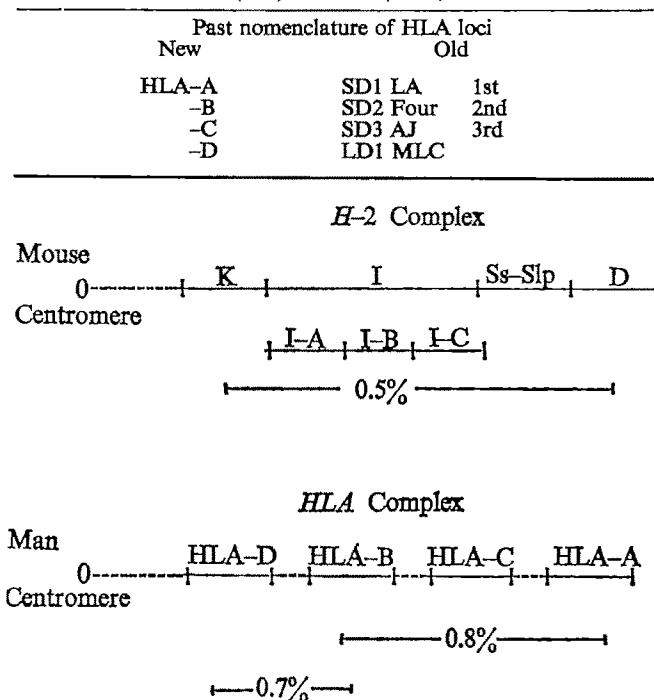
It is important to have some feel for the size of the MHC and hence a crude estimation of the number of possible genes involved. The MHC covers a length of DNA equivalent to 0.5 recombination units in the mouse¹ and greater than 1.0 in man^{5,6}. The low frequency of recombinants between the different loci in the MHC means that in most cases offspring inherit from each parent all the alleles found on one of the parental chromosomes. Such a set of alleles is known as a haplotype. It is not known how much of the DNA in the MHC codes for protein. The amount of DNA in a piece of chromosome of this size could, however, code for as many as 2×10^3 polypeptide chains⁷. It is important to remember that each region probably contains several genes. When more than one property is assigned to the products of a particular region then it does not necessarily follow that these properties belong to the same gene product. In time each region may be further divided as new recombinants are identified.

The classical histocompatibility antigens (CHAGS)

Historically the first products of the MHC to be characterised were those from the K and D regions in the mouse and the HLA-A and -B loci in man^{1,4}. The products from these regions have many biochemical and functional properties in common and seem to form a group of related molecules, which we shall call CHAGS.

The CHAGS can be detected by using antisera induced by the introduction of histoincompatible cells into recipients. In both species the antigens detected by such antisera are found on all tissues except possibly early embryonic tissue and mature human red blood cells. Individual cells

Fig. 1 Diagrammatic representation of the MHC of mouse (H-2) and man (HLA).



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from a heterozygous animal express the alleles from both chromosomes while individual sperm seem to carry the CHAGS and other MHC gene products coded for by a single chromosome^{9,10}.

Using different antisera people quickly realised that there were many alleles at each locus. In the mouse each allele of the K and D locus is described by a series of numbers representing individual determinants which fall into two categories; a private specificity which is generally unique to that allele and a set of public specificities which occur in more than one allele. Both types of determinants are on the same polypeptide chain¹¹⁻¹³, although there may be possible exceptions to this rule^{14,15}.

In humans the alleles are typed by using specially selected antisera which probably recognise the equivalent of only the private specificities in the mouse. Consequently each allele is described by one number, for example, HLA-A1, HLA-B5. There is increasing evidence for the existence of public specificities in man which may account for some of the anomalous patterns of reaction found with many anti-HLA antisera^{16,17}, for example 4a and 4b. It is not known if the present typing methods for HLA-A and HLA-B private specificities are adequate for transplant and disease association studies or whether typing for public specificities should be included. This is particularly important as private specificities in mouse and man are not always associated with the same public specificities respectively^{18,19}. It is worth noting that some human specificities of a public type are found in other species²⁰.

By using antisera to the CHAGS to monitor the purification, it has been possible to isolate membrane proteins carrying the corresponding antigenic specificities²¹. In both human and mouse these are glycoproteins of molecular weight ~ 45,000. The allelic forms differ in the amino acid sequence and it seems that the carbohydrates are not responsible for the allelic variants^{21,22}. Amino acid sequence data of the N-terminal ends²³⁻²⁶ have confirmed the structural homology of the K and D region CHAGS previously suggested by shared public specificities²⁷. There is also marked sequence homology between HLA-A and HLA-B N terminal fragments^{28,29}, although as yet the serological evidence for such a homology is weak^{30,31}.

There is structural homology not only between the CHAGS from one species but also between the N-terminal fragments of the CHAGS from different species. This homology lends weight to the suggestion that one is working with homologous systems; but the interspecies homology is less marked than that homology within species, and this raises interesting evolutionary questions. It would seem that speciation preceded duplication to form multiple loci. Ways in which this may have been achieved have been discussed at length elsewhere³².

The human and mouse CHAGS have other features in common. Both are found on membranes non-covalently associated with β_2 microglobulins³³⁻³⁶, remarkable proteins of molecular weight 12,000, which have 30% homology with an immunoglobulin domain^{37,38}. In both animals it seems that the carboxy-terminal end of the CHAGS is involved in attaching the molecules to membranes. A carboxy-terminal fragment of about molecular weight 8,000 is lost if proteolytic enzymes are used to solubilise the molecules from the membrane. The previously isolated polymeric forms of purified human CHAGS are now thought to be artefacts caused by the extraction procedures^{39,40}. Further it has been shown that the allelic products of each locus move independently on lymphocyte membranes^{41,42}. Consequently the molecules only interact with each other very weakly if at all on the surface of cells.

The independent movement of certain determinants on human lymphocytes supports the suggestion that there is a third locus in man, HLA-C, the products of which are

like HLA-A and HLA-B in their tissue distribution and association with β_2 -microglobulin^{35,43}. The relationship between HLA-C and the other two loci (HLA-A and HLA-B) is not yet known, particularly as HLA-C seems to be weakly immunogenic compared to HLA-A and B⁴⁴. The association of the CHAGS with β_2 -microglobulin has led to much premature speculation on the possible evolutionary relationship between CHAGS and immunoglobulin. The structural homology between β_2 -microglobulin and immunoglobulin is such that it is reasonable to assume that β_2 -microglobulin has the same overall three-dimensional structure as a variable region or constant region domain⁴⁵. Immunoglobulin domains readily interact to form dimers, and it has been suggested that the binding of β_2 -microglobulin to the CHAGS occurs by way of a similar mechanism. If this is so there should be a region with homology to an immunoglobulin domain in the sequence of the CHAGS. So far there is no evidence for this though the sequence data are still very limited.

Genetic properties of the CHAGS

We want now to discuss the exceptional genetic properties of the CHAG alleles, which set this system apart from most other known allelic systems. Within a population certain pairs of alleles, one from the A and one from the B locus, occur as haplotypes with a frequency which is greater than or less than that expected if the alleles were randomly associated in the population. This is known as linkage disequilibrium. For example, the haplotypes A1, B8 and A3, B7 are more common in the Caucasian population than would be expected from the frequencies of the individual alleles. Linkage disequilibrium can occur when there has been insufficient time for equilibrium to be established in the population concerned. It is generally thought that this is not the case for the CHAGS in most human populations⁴⁶. Alternatively, linkage disequilibrium can be caused by selective pressures operating in the population to hold certain pairs of alleles on the same chromosome. In different populations the HLA alleles occur with different frequencies and different haplotypes can be found in linkage disequilibrium in different races⁴⁷, which suggests that the selective pressure to establish linkage disequilibrium may be different in each race. Further these pressures need not operate on the CHAGS themselves but on genes on either side of them.

Another unusual feature is that most of the twenty or so alleles at the A and B loci have similar frequencies to each other in the Caucasian population. Selective pressures are required to account for this balanced and extensive polymorphism, for example through favouring heterozygosity^{7,48}. If selection operates through resistance to diseases then such pressures may no longer apply to populations treated by modern medical techniques. In Caucasian families now, segregation of HLA-A and HLA-B obeys the Hardy-Weinberg equilibrium^{49,50}, possibly reflecting the absence of selective pressures in western societies. It is interesting that in at least one population (Tuaregs) there is evidence for heterozygote advantage⁵¹. Because of linkage disequilibrium in the MHC the selective pressures to establish balanced polymorphism of the CHAGS may operate on linked genes, for example, the immune response genes (see later).

In this connection it has been suggested that heterozygosity of H-2 in wild mice may be promoted by the linked *T/t* complex⁵², which is said to control surface structures important in development⁵³. There are lethal recessive alleles (*t*) of several loci in this complex. Two unusual properties of these *t* alleles could lead to extensive heterozygosity at H-2. First, male mice carrying *t* alleles transmit the mutant form to their offspring with an exceptionally

high frequency. Second, many of the *t* mutants suppress recombination over a length of chromosome extending to the H-2 complex. This provides a mechanism by which H-2 heterozygotes are favoured as *t* mutants are lethal in the homozygous state. The *t* mutants are common in wild mice and can be found in as many as 40% of certain populations¹.

There is no evidence that a similar *T/t* complex exists in man, in fact there is evidence to the contrary. The properties of the *t* alleles are such that one would expect to see diversion from Mendelian segregation of the MHC. This has not been observed, at least in Caucasians. It thus seems likely that if the *T/t* complex does exist in the human then it does not bear the same relationship to the MHC as in the mouse. Alternatively the human *T/t* complex may not have the same unusual properties as the mouse complex.

Cytotoxic T lymphocytes and the CHAGS

Although in the past various biological functions have been suggested for the CHAGS^{7,48,52}, there has been little evidence to support any of them. Recent experiments have shown that the CHAGS are in some way involved in the interaction of cytotoxic T cells with their targets. In mouse and man reaction against a foreign MHC, for example in a mixed lymphocyte reaction, generates cytotoxic T cells⁵⁴⁻⁵⁶. In general the generation of such cytotoxic T cells requires cooperation between different populations of T cells: helper T cells which respond to differences in the I region (or HLA-D), and the cytotoxic T cells which respond to differences in the CHAGS^{59,60}. The cytotoxic T cells generated in a primary reaction of this type will kill target cells carrying the CHAGS to which they have been activated. In mice, for example, T cells activated to the H-2^a antigens will kill only target cells carrying an H-2^a CHAG at either the K or D region: the nature of the I or S regions is unimportant. Thus it seems that the CHAGS are the antigens with which such cytotoxic T cells interact. This restricted specificity of cytotoxic T cells must be a consequence of either a restricted range in specificities of the receptors for antigens on cytotoxic T cells (which seems unlikely) or a restriction imposed by the nature of the assay for cytotoxic function. In this context it is interesting that target cells lacking CHAGS can be killed by cytotoxic T cells only in the presence of concanavalin A (con A)⁶¹ a substance which may bypass the structures involved in normal recognition mechanisms.

It has been suggested that allogeneic responses are biologically important, for example, to prevent transmission of tumours between individuals⁶². However, the successful survival of inbred strains of animals makes a biologically important role for such reactions unlikely and they probably occur only in artificial situations. Consequently the fact that the antigenic targets of cytotoxic T cells generated in an allogeneic reaction are the CHAGS is of little general significance. In mice, however, it has also been shown that in reactions of cytotoxic T cells to surface antigens other than CHAGS, the nature of the K and D region gene products on the target cells is still vitally important. Cytotoxic T cells specific for a variety of different antigens have been used in these experiments including, (1) viral antigens on virus infected cells⁶³⁻⁶⁶, (2) histocompatibility antigens other than the K and D region molecules^{66,71}, and (3) haptens on chemically modified cells⁷²⁻⁷⁴. In each case the cytotoxic T cells are lytic only if the target to be lysed carries the specific antigen and also the same K or D region molecules as the original stimulating cells. For example cytotoxic T cells from H-2^k mice infected with vaccinia virus will kill H-2^k fibroblasts if they are also infected with vaccinia, but not virus-infected H-2^b cells. But because of the partial homology between certain CHAG alleles (as reflected in their shared public specificities) it is not sur-

prising to find a certain amount of cross reactivity in some cases^{66,69}. (This is also seen with cytotoxic T cells generated by an allogeneic reaction^{75,76}.) It is not at all clear why both stimulating and target cells for cytotoxic T cells must express the same K or D region molecules. It is even possible that the K or D region molecules involved in some of these cytotoxic reactions are not the CHAGS but products of closely linked genes^{57,58,67,68,75}. Further, it is possible that the effects seen with different antigens, although similar in appearance, are different in origin. This may be particularly true for hapten modified cells.

Two explanations have been put forward to explain the above phenomenon. The first is based on the proposition that the specific antigens cause a modification of the CHAGS. According to this theory, which is known as the "altered self" hypothesis, the modified CHAGS are the structures that are recognised by the cytotoxic T cells. For example, virus proteins on the surface of cells might combine with the CHAGS and the complex become the new antigenic determinant. Alternatively the viral proteins might interact with the CHAGS, causing the CHAGS to alter their shape and hence become foreign antigens. It is hard to imagine in molecular terms how these alterations are brought about, particularly when the specific antigens concerned are the minor transplantation antigens rather than viral proteins. The fact that about 10% of unprimed T cells react to all the antigens in a single foreign MHC⁷⁹ has led to the suggestion that T cell receptors are designed to react with foreign MHC antigens. This would support the "altered self" hypothesis, particularly if the modified CHAGS were to resemble the CHAGS found in other members of the species⁸⁰. It is not known, however, if unprimed cytotoxic T cells show a similar high percentage of reactivity to a set of allogeneic CHAGS.

The second explanation proposes that cytotoxic T cells require dual recognition of target cells through (1) specific antigen and (2) CHAG determinants to kill the targets. This hypothesis takes various forms depending on the nature of the proposed recognition of CHAG determinants. It now seems unlikely that this is through a requirement that CHAGS on cytotoxic T cells should be identical to those on their target. Thus H-2^k cytotoxic T cells prepared from H-2^k-H-2^d chimaeric mice infected with lymphocytic choriomeningitis virus, killed with equal efficiency H-2^k and H-2^d virus-infected target cells. Supporters of this type of dual recognition hypothesis can still argue that the important requirement is that the target cells should carry at least some of the CHAGS which were present at a critical stage of the maturation of the cytotoxic cell. The cytotoxic cells in a chimaeric animal would mature in the environment of H-2^d and H-2^k cells and hence kill both types of target.

Current opinion favours a dual recognition hypothesis in which cytotoxic T cells recognise their targets by two independently coded receptors, one of which reacts with the specific antigen and the second with a CHAG determinant⁸¹. By this hypothesis the restriction of cytotoxic T cells recognition lies in the narrow range of structures (CHAG determinants) with which the hypothetical second receptor reacts. It is difficult to visualise a molecular mechanism by which a family of receptors reacts specifically with CHAG determinants and no other antigens.

A third form of the dual recognition model hypothesises that a cytotoxic T cell recognises its target through a single receptor which reacts with specific antigen and, through cross reaction, with a CHAG determinant on the target. Some receptors on a cytotoxic T cell would react with the target through specific antigen while other identical receptors on the same cell would react with CHAG determinants on the target. This hypothesis implies that such dual interaction is not only important for cytotoxic

function, but necessary for the proliferation of cytotoxic T cells so that only clones carrying the appropriate cross-reacting receptors are selected.

In summary, much is still to be learned about T-cell recognition and cytotoxicity and no wholly satisfactory explanation exists for the restriction seen in these systems. It is not yet known if such restriction applies to cytotoxic T cells in man.

Introduction to the I region

The I region of the mouse and the region around the HLA-D locus in man have much in common. Both were initially studied by using a mixed lymphocyte reaction (MLR) as a means of detecting antigenic differences. (The so-called lymphocyte defined or LD antigens.) It is only recently that serological techniques have been applied to detect allelic forms of proteins coded by these regions. In the mouse it has been shown that these gene products are also involved in graft rejection and cell interaction; between T cells and B cells for the production of antibody, between different populations of T cells to generate suppressor cells, and between macrophages and T cells. The other important immunological phenomena associated with the I region are the immune response genes (Ir genes) which control the response to specific antigens. Ir genes have been found associated with the MHC in all species in which they have been looked for but so far in man the evidence for them is more circumscribed, mainly because immunisation with the appropriate antigen would be unethical. We will first discuss the biochemical properties of the products of the genes from this region and then their different biological properties.

Use of antisera to study the I region

Antisera specific for I region antigens have revealed several different antigenic systems on mouse B lymphocytes²². The antigens are also found on macrophages, sperm, epidermal cells^{22,23} and on some T cells, particularly T cells which have been activated by con A (refs 84-86). They are notably absent from erythrocytes and platelets. The different antigen systems are coded by different subregions of the I region, I-A, I-C and I-E and so on^{22,27}. The alleles of each system are described by a series of numbers representing individual determinants and studies on wild mice suggest that there may be private and public specificities as found on the CHAGS²⁴. In the inbred strains of mice not all the specificities have yet been assigned to I subregions and new specificities and I subregions are still being discovered. The term Ia antigens refers to all antigens associated with the I region. It is important to stress that the molecules carrying the numbered specificities described above represent only a part of the Ia antigens. For want of a better term we shall call the numbered Ia specificities the B-Ia antigens.

Membrane molecules have been isolated which carry B-Ia antigenic determinants²⁵. The specificities coded by genes in the I-A and I-C subregions are carried on different molecules^{25,26,27}. On the other hand the specificities determined by one allele from either subregion have so far been found on the same molecule²⁷. The molecules carrying the B-Ia specificities are glycoproteins each of which consists of two different polypeptide chains held together by disulphide bridge(s). It is not known if both chains carry antigenic determinants coded for by the I region. The molecules carrying the B-Ia determinants are not associated with β_2 -microglobulin on cell membranes.

There is a system similar to the mouse B-Ia antigens on human B lymphocytes²²⁻²⁷. Genes coding for these antigens seem to lie at several loci, at least one around the HLA-A locus and a second to the left of the HLA-B locus. In fact

one serum seems to show complete concordance with HLA-D typing²⁸, though this is not generally the case. This work is still at an early stage and it is not yet known how many loci are involved or the extent of the polymorphism. As yet there is no unified terminology for this system of antigens and although it has not been conclusively shown that they are analogous to the mouse proteins we shall call them the human B-Ia antigens. The existence of strains of mice which differ only in the I region and are otherwise genetically identical, makes it relatively easy to define mouse Ia antigens. In humans the absence of such defined genetic differences makes it very difficult to be certain that any anti-B-Ia antisera do not also contain activity to other unrelated antigens. The molecules carrying the human B-Ia determinants have not been definitely identified but from knowledge of the mouse antigens obvious candidates are the glycoproteins which contain two polypeptide chains which can be extracted from human B-cell membranes^{29,100}.

I region and the mixed lymphocyte reaction (MLR)

As we have already mentioned, differences in the I region evoke a 'strong' MLR²². This is measured by following the stimulation of DNA synthesis, after mixing lymphocytes from two different strains or individuals¹⁰¹. The cells which respond and proliferate are T cells¹⁰². Other regions in the MHC can cause an MLR but the response is usually much smaller¹⁰³. (In the mouse the M locus unlinked to the MHC can also cause an MLR¹⁰⁴.) The cells which are particularly adept at stimulating a response in an MLR are B-lymphocytes. Other cells including T cells, sperm¹⁰ and possibly epidermal cells¹⁰⁵ can also stimulate an MLR. In the mouse anti-B-Ia antisera directed against the stimulating cells will readily block their capacity to stimulate an MLR¹⁰⁶. Although it is notoriously difficult to interpret such experiments, it is natural to suggest the molecules carrying the B-Ia specificities described in the previous section are the molecules which are involved in stimulating the T cells which respond in MLR.

In man differences at the HLA-D locus provoke a strong MLR^{107,108} which has been exploited to type cells for this locus^{97,109}. The HLA-D locus shows balanced polymorphism but as yet the extent of the polymorphism is not known. The idea that B-Ia antigens evoke an MLR is again supported by the fact that one anti-human B-Ia antiserum reacts only with cells typed as HLA-D-DW3 by MLR⁹⁸. More usually, however, B-Ia antisera react not only with all cells with a particular HLA-D allele, but also with some cells carrying different HLA-D alleles; and some sera show no concordance with HLA-D at all⁹⁷. This type of result is difficult to interpret as HLA-D typing by MLR and serological typing may well have different sensitivities, and further because there is increasing evidence for cross reactivity between different HLA-D alleles⁹⁷.

I region and cell cooperation

The interactions between various cells of the immune system seem to depend on the compatibility of the interacting cells at the I region. Until recently, however, it was not clear whether that was because I region products played an essential part in cell cooperation, or simply because of an allogeneic reaction which abrogated cooperation. By the use of several techniques for eliminating the problem of allogeneic reactions, it has recently become possible to clarify the issue and it seems that I region products play an essential part in cell-cell cooperation, but that I region compatibility is not always necessary.

For example, cooperation between helper T cells and B cells in the production of antibody does not occur when the T and B cells are incompatible at the I region¹¹⁰⁻¹¹²,

unless an allogeneic reaction is prevented by the use of tolerant T cells^{113,114} or T cells selected for their inability to respond to I-region differences¹¹⁵. In interactions between antigen, macrophages and T cells it seems that I region products may play a part in recognition¹¹⁶⁻¹¹⁸. Helper T cells will produce a secondary response to an antigen-macrophage complex only if the antigen is presented on a macrophage carrying the same I region products as those on the priming macrophage^{117,118}. (In this case the effect of histocompatibility reactions have not been fully investigated.) It is now possible to circumvent the complications arising from allogeneic reactions altogether by using soluble T-cell factors to study the control of the immune response.

These factors fall into two classes. First, there are antigen specific factors which bind to their corresponding antigens and lead to specific responses¹¹⁹. Second, there are non-specific factors which are induced by antigens¹²¹, mitogens (O. Sjöberg, unpublished) or an MLR^{122,123}, which enhance an antibody response to certain antigens¹²⁴. The antigen specific helper factor is not classical antibody and at least part of it must be coded for by genes in the I region since it reacts with anti-Ia sera¹²⁵. Treatment of B cells with anti-Ia antiserum prevents the cells from absorbing factor, which implies that at least parts of the B-cell acceptor sites for the T-cell factor are also coded by genes in the I region¹²⁶. Thus two different, but interacting, products of the I region are involved in T-B-cell cooperation. Preliminary evidence suggests that the acceptor molecule carries the B-Ia determinants and it may be the acceptor molecules that are responsible for stimulating an MLR. All that is known of the antigen specific factor is that it is a glycoprotein of molecular weight about 50,000. So far no allogeneic barrier has been found for the helper factor¹²⁸ and indeed mouse factor will work with human lymphocytes¹²⁷.

A second similar, but not identical, antigen specific factor is involved in T cell-T-cell interactions in the generation of suppressor T cells¹²⁹⁻¹³¹. The factor binds to antigen, reacts with anti-Ia sera but not anti-immunoglobulin sera and has a molecular weight of approximately 50,000. The factor is absorbed by T cells but not B cells¹³². This suppressor factor shows certain allogeneic barriers in that factor from one strain of mice will not react with all other strains. It has been suggested that the ability to absorb the suppressor factor is also related to I region products which mean that the I region is involved in two systems of interactive gene products important in regulation of the immune response¹³². It is clear that suppressor and helper systems are different but it is quite possible that they share genetic elements.

These antigen specific factors are likely to be closely related if not identical to the actual receptors for antigen on T cells. It has so far proved extremely difficult to characterise unambiguously antigen receptors on T cells, particularly as T cells will absorb serum antibody^{133,134}. By using antisera which react with idiotype determinants on the variable region of immunoglobulin heavy chains it is possible to obtain evidence that an antigen receptor on T cells contains at least parts of heavy chain variable regions^{135,136}. To date there is no evidence that the antigen specific factors described above contain any part of immunoglobulin molecules, including the variable regions. Other factors which may be important in the regulation of the immune response, do react with anti-immunoglobulin antisera^{137,138}. There is no simple way to explain these apparent contradictions concerning the nature of the T-cell receptor for antigen but it is clearly important to find out the exact chemical nature of these antigen specific factors.

There are several ways that lymphocytes can be provoked into producing a nonspecific factor which can enhance the response of B cells to particular antigens. One factor with this activity seems to be a complex of β_2 microglobulin and

Ia antigens^{133,139} though other functionally similar factors produced differently do not react with anti-Ia sera (ref. 140 and A. Schimpl and E. Wecker, personal communication). The relationships of these nonspecific factors to each other and to the specific factors are not known.

Immune response genes

The discovery of immune response (Ir) genes in the MHC is extremely important and has acted as the catalyst for much of the work on the I region. It is known that certain individuals within a species fail to respond to a specific antigen and this deficiency maps within the I region. So far, in different mouse strains, defects have been found in the response to more than 40 antigens. The Ir genes controlling these responses lie in all three subregions of the I region. In every case the absence of response is recessive—that is, response is dominant^{141,142-143}. Further analysis of these defects has revealed that for at least some of these antigens there exist at least two Ir genes which can complement each other^{129,144-149}. Thus in some cases the hybrids between two strains which do not respond to a particular antigen may themselves respond. The existence of complementation groups suggests that at least two types of I region product are necessary for an immune response. The antigen specific factor and its acceptor described in the previous section are obvious candidates as products of a complementing gene system and indeed with certain antigens there is some evidence to support them^{129,150,151}. Similarly it has been found that there are at least two complementation groups for the generation of suppressor T cells^{132,152}. I region products have been shown to be involved in the regulation of the response to a wide variety of antigens though Ir gene defects are only seen for antigens which carry a restricted number of determinants. This suggests that responses to complex antigens involve the products of several complementing systems. Defects in a component of one system need not affect the activities of the others.

There are many immune response genes which are not associated with the MHC, some of which have been mapped to particular loci. Recently it has been shown that the products of one of these other Ir-genes is important for the interaction of antigen specific helper factor with B cells¹⁵⁰.

The complement system and the MHC

There are genes in the MHC which affect the complement system, a series of serum proteins activated characteristically by antibody-antigen reactions. These genes may function two ways. First, as structural genes which code for certain of the complement components. The most convincing evidence for this comes from linkage studies of allelic variants of certain complement components. Second, there may be a gene or genes which control the level of certain complement components. The first indication that genes affecting the complement system lay within the MHC came from work on the mouse Ss-Slp system. Ss and Slp determinants are found on two serum proteins whose levels are affected by sex hormones and by genes in the H-2 region, now mapped between I and D¹⁴¹. Anti-Ss antiserum was shown to inhibit complement mediated haemolytic activity of mouse serum¹⁵⁴ and it now seems that Ss protein is mouse C4 (refs 155 and 156).

In man and rhesus monkey several allelic forms of factor B can be detected by electrophoresis. The genes coding for these variants are linked to the MHC^{157,158}. The structural gene in man for factor B has been tentatively placed to the left of HLA-B¹⁵⁹. Since factors B and C2 have analogous properties in the alternative and classical complement pathways and very similar physicochemical characteristics it has been suggested that the structural genes for these proteins are tandem duplicates¹⁶⁰. Recent evidence suggests that the C2 electrophoretic variants are indeed

linked to the MHC¹⁶⁰. Structural genes of this type, lying within the MHC, may be useful for understanding the pressures which generate balanced polymorphism at other loci. If these pressures operate on the MHC as a whole one would expect to see balanced polymorphism in complement components coded for by MHC genes. This would only be true if such complement components were able to accommodate sufficient structural changes and remain functional. So far only four functional alleles of factor B have been found, two of them uncommon¹⁶¹.

In addition to the structural genes for complement components in the MHC there may be genes which control the serum levels of these components. The Ss-Slp system may contain this type of gene. Deficiencies of particular complement components could represent either deletion of structural genes or malfunction of a controlling gene. In man C2 deficiency is linked to HLA in a number of family studies and seems to be in linkage disequilibrium with the haplotype A10-BW18¹⁶²⁻¹⁶⁵. Deficiencies of C4 and C8 have also been found to be linked to particular HLA haplotypes although only one family has been studied in each case¹⁶⁶. If these deficiencies represent malfunction of genes controlling the levels of complement components then it is possible that only one such gene exists in the MHC and that this gene may also be responsible for the observed MHC influence on levels of the receptor for complement components on lymphocytes¹⁶⁷. Naturally not all the genes for the complement system lie within the MHC C3 and C6 electrophoretic variants in man^{168,169} and Clr (man)¹⁷⁰ and C5 (mouse)¹⁷¹ deficiencies are unlinked to the MHC.

MHC and disease

Many diseases in humans have an association with particular CHAGS¹⁷². These associations can be divided into four types, each of which may have a different origin.

(1) The only very strong association is that of HLA-BW27 with ankylosing spondylitis and a related group of diseases. About 95% of Caucasian patients with ankylosing spondylitis have this allele¹⁷³⁻¹⁷⁵. It is likely that the cause of this strong association lies in a property of the BW27 protein itself, particularly since it is found in other racial groups^{176,177}. There are various ways in which CHAGS might directly influence the susceptibility to disease. For example, they could function as receptors for viruses or they may resemble certain pathogens so closely that the animal is unresponsive to that pathogen through self-tolerance^{7,92}. The absence of BW27 in a few patients with ankylosing spondylitis is still compatible with the theory that the BW27 protein is directly involved in susceptibility to the disease. Ankylosing spondylitis is a multifactorial disease, and in the BW27 negative patients there may be an excessive accumulation of the other factors involved.

(2) There can be weaker, but still significant associations of diseases in the general population with individual CHAGS, for example thyrotoxicosis and some other autoimmune diseases with B8 (ref. 178) and psoriasis vulgaris with BW17 (ref. 179). A weak association would be expected if the genes responsible for increased susceptibility were closely linked to the CHAGS and remained with particular CHAG alleles through linkage disequilibrium. However, these diseases could be multifactorial and the CHAGS themselves may be one of the factors directly involved in increased susceptibility. In this case as with ankylosing spondylitis one would expect to see the same CHAG allele involved with the disease in studies of different races.

In general if immune response genes are the reason for the observed associations, an allele leading to an absence of response would only have a deleterious effect in homozygotes as response is dominant. On the other hand, alleles which lead to an excess, or normally unwanted, response

would be dominant in heterozygotes. The diseases in the categories discussed above are in general not found with greater frequencies in MHC homozygotes. Consequently non-responder alleles are unlikely to be responsible for disease association. Many of the diseases in these two categories have an auto-allergic element which would be expected if the deleterious Ir-genes lead to an autoallergic response.

(3) A third category of association of CHAGS with disease are those cases in which there is a higher frequency of particular CHAGS among individuals with greater resistance to particular conditions. For example, there is a higher than average proportion of HLA-A2 positive individuals among long term survivors of acute lymphocytic leukaemia¹⁸⁰. This association may again be due to a rare responder allele of an immune response gene linked to HLA-A2 but in this case the response is beneficial. Linkage to beneficial responder alleles could also be responsible for the observed low proportion of particular CHAGS among individuals suffering from certain conditions¹⁸¹ (for example HLA-BW15 and chronic viral hepatitis)¹⁸².

(4) Finally, it is possible to find a particular CHAG associated with a disease within a family though not in the general population. For example in ragweed allergy sensitivity is inherited in particular families together with an MHC haplotype¹⁸³. In this situation the gene involved is unlikely to be a CHAG gene but some linked gene, again probably an immune response gene.

In all cases if the association of disease to CHAGS is through linked Ir genes it is sensible to look for associations with other markers in the MHC. Several studies have used the HLA-D locus for this purpose^{93,184}. In many cases association with HLA-D is stronger than with CHAG. This would be expected if the important genes were the Ir genes providing the relative distribution of MLR and Ir genes is the same in man and mouse. However, there are many immune response genes and their exact distribution is not known, even in the mouse, so that diseases most strongly associated with the CHAG could in fact be explained by close linkage of an Ir-gene. It is also possible that several genes in the MHC influence susceptibility to a single disease. Under such circumstances one would expect to see the disease associated more strongly with a haplotype than with individual markers¹⁸⁵.

Finally, in a few cases the association of disease with the MHC is probably a consequence of defects in the complement genes. For example, systemic lupus erythematosus is found associated with C2 deficiency and hence with the haplotype A10-BW18¹⁸⁶.

Coda

It should be clear from what we have written that there is still much to be learnt about the MHC and it is probably premature to attempt a rational synthesis of all the facts. Phenomena which need to be explained are the balanced polymorphism at some loci, linkage disequilibrium, and the biological function of the CHAGS and determinants responsible for an MLR. In addition, the implications of the maintenance of these immunologically important genes in one complex during evolution are not understood.

It is possible to construct a moderately coherent explanation of the facts with the products of the immune response genes playing a central role. As these genes are codominant and it is usually desirable to minimise the number of determinants to which an individual cannot respond, there will be heterozygous advantage. This is particularly true if the number of immune response genes approaches the limit for stable tandem duplication. This accounts for balanced polymorphism of immune response genes which could be transmitted to the CHAGS through linkage dis-

equilibrium, provided that CHAGS can accommodate many amino acid replacements without losing whatever biological function they have. The balanced polymorphism of the CHAGS might simply be a consequence of their linkage to the Ir genes and have no relationship to their own biological function.

The properties of the products of the I region could also account for linkage disequilibrium. The fact that products from separate immune response loci in the MHC have to react with each other to control the immune response would lead to a selective advantage to the transmission of pairs of alleles together as a haplotype.

The above synthesis does not explain why genes for complement components are found in the MHC, nor does it cope with the particular attention T cells seem to pay to the CHAGS in all cytotoxic reactions. An alternative explanation of polymorphism is that the CHAGS are involved in developing T-cell immunity to viruses and other pathogens¹⁸⁶. It has been shown that heterozygosity of the CHAGS on virus infected target cells increases the efficiency of T-cell cytotoxicity. There are many other ways by which pathogens can be recognised and dealt with *in vivo*, which will reduce the pressure for heterozygosity of the CHAGS. Although this model ascribes a biological function to the CHAGS it does not explain linkage disequilibrium or their association with Ir genes, nor is there at present any clear understanding of the mechanism by which antigens on virus-infected cells become associated with the CHAGS.

If the CHAGS act as the actual receptors for viruses, it is possible to give an alternative explanation for linkage disequilibrium¹⁸⁷. Any individual with a receptor for a particular viral pathogen will be at risk especially if that person does not carry the appropriate immune response gene, for resistance. Thus pairs of alleles will be encouraged to travel together—a CHAG allele and the appropriate immune response gene. Linkage disequilibrium in the intervening piece of DNA will ensue. There may be other structures in the MHC which act as virus receptors¹⁸⁸.

It is obviously important to know if the nature of the genes clustered together in the MHC is of some significance and has therefore been conserved in evolution. It is at present hard to imagine why particular genes are clustered together. It has been suggested that the MHC has evolved by tandem duplication from the *T/t* complex¹⁸⁹. The possible similarities between molecules from the *T/t* complex and H-2 complex lend weak support to this unlikely idea. It is obviously too early to say what relevance the *T/t* complex may have to the MHC and indeed it is really too early to promulgate any further unsubstantiated speculation.

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articles

Radio-echo layers and the recent stability of the West Antarctic ice sheet

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A new method for studying polar ice sheets confirms the generally accepted concepts of ice sheet flow and finds that a region near the ice crest of the West Antarctic ice sheet has been stable for ~ 30,000 yr.

A CHANGE in the mass or configuration of the large continental ice sheets could affect world sea level, alter the average albedo of the polar regions, and change the climate^{1,2}. To determine whether the ice sheets have changed, are changing, or are likely to change in an im-

portant way, and to allow the interpretation of palaeoclimatic results of studies of deep cores it is necessary to understand the history and dynamics of ice sheet flow.

One way to study the present-day balance of an ice sheet is to compare data on ice movement with surface mass-balance (new snow accumulation) data. This can be done by comparing measured changes in the elevation of markers in the surface with local surface mass balance^{3,4}, or by comparing the horizontal flow of ice with the rate of up-glacier replenishment by new snowfall⁵⁻⁹. In general, however, the interpretation of such studies is difficult because

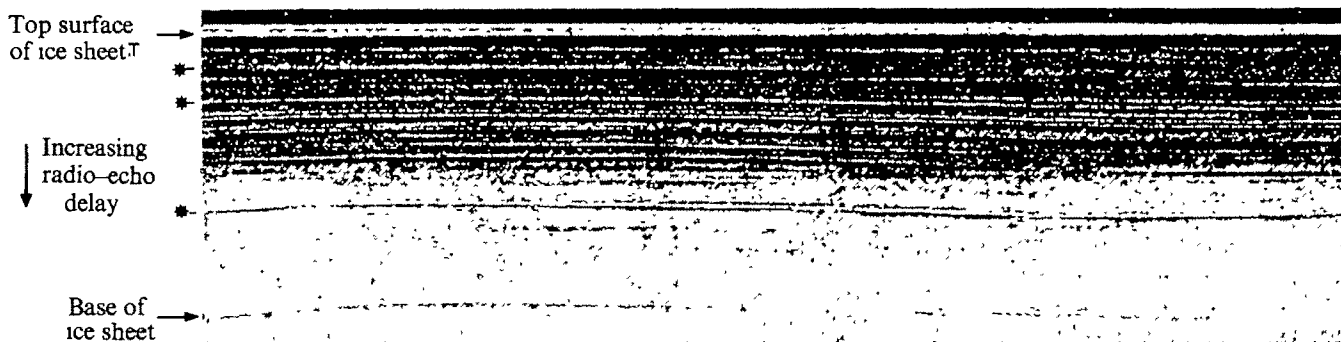


Fig. 1 Example of radio-echo record. A profile ~ 16 km long is represented. This example is located ~ 70 km from Byrd Station (Fig. 2), where the ice is $\sim 2,500$ m thick. Stars indicate the layers selected for analysis.

of uncertainties in measurement or because deep ice velocities are not known. A more satisfactory method for determining the health of an ice sheet is needed.

The recent histories of the Greenland and Antarctic ice sheets have been discussed using profiles of ice temperature¹⁰⁻¹², total gas content¹³⁻¹⁵, and ratios of the stable isotopes of hydrogen or oxygen¹⁶⁻¹⁸. Although very valuable, none of these techniques can distinguish clearly between changes in the form of the ice sheet and climatic changes.

A new approach is described here for studying the present and past dynamics of large ice masses. The method combines measurements of surface mass-balance, surface strain-rate, and the form of internal layering in the ice sheet determined by a radio-echo sounding technique.

Method

During the austral summer 1974-1975, radio-echo sounding flights were conducted in the Antarctic¹⁹. Equipment, designed and built by the *Danmarks Tekniske Højskole* (Lyngby, Denmark), and operated by members of the Scott Polar Research Institute (Cambridge, UK), was flown in an LC-130 (Hercules) aircraft by the US Navy as part of the United States Antarctic Research Program. Figure 1 is an example of a record from these flights: it shows radio reflections not only from the surface and base of the ice sheet, but also from within the ice mass. Many of these internal layers can be traced horizontally for >170 km. It has been suggested that the internal reflections are associated with sedimentary variations in density or impurity content, or old refrozen melt layers²⁰⁻²³, with certain annual layer spacings, or with horizons at which the mean ice crystal orientation changes²¹.

Flights were made along and perpendicular to the axis of the Byrd Station Strain Network (BSSN), which is up-glacier from Byrd Station, Antarctica (Fig. 2). Surface velocities and surface mass balances have already been measured along the BSSN^{9,24}, and using these data, it is possible to calculate the depths of ice layers that were deposited at different times in the past. The form of these buried layers is compared here with the internal layering revealed by the radio-echo sounding.

The model

A very simple model for the flow of the ice sheet is used. It is assumed that the strain rates, as measured at the surface, are constant throughout the thickness of the ice mass, but they vary in horizontal directions. The ice sheet is initially assumed to be in a steady state (with ice thicknesses and velocities independent of time), but possible departures from the steady state can also be discussed. The model

cannot adequately describe the flow of ice over and around variations in the bed, but is conceptually simple and adequate for present purposes.

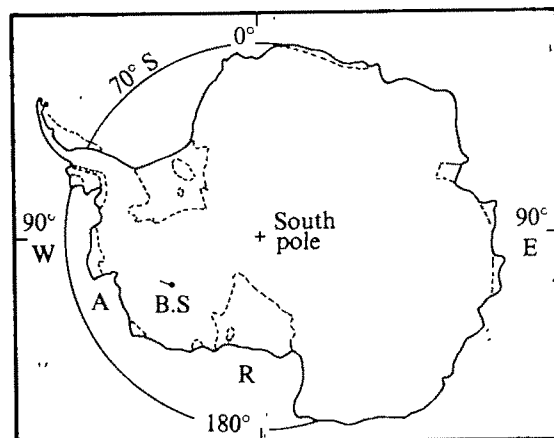
According to the model, the horizontal velocity of a vertical column of ice is the same at all depths, and variations in the bed of the ice sheet do not generate strains within the ice sheet except those that are measured at the surface. A layer of snow deposited at the surface, after compaction to ice, becomes buried by later accumulation, is thinned by the extending flow, and is carried down-glacier as the ice sheet flows towards the sea. The depth of a layer of any age may thus be calculated, and unlike the rather similar models of Robin and others²², and Harrison²¹, ice thickness and bottom topography are not used in the computation.

The model results and the radio-echo results are independent and the model was developed before the radio sounding flights were conducted. The strain rate and velocity, as measured at the surface, are, in part, physically determined by ice thickness and bottom topography, so that some correlation between calculated buried layer form and the base of the ice sheet is to be expected for larger scale features of the relief.

Model—layering agreement

Figure 3 shows a profile of the ice sheet along the axis of the BSSN. The data for the depth calculations come only from surface measurements along the BSSN. The calculated isochrons and the measured radio-echo layering show agreement that is much too good to be coincidental, confirming the results of a similar study made along a flowline in northern Greenland²². This shows that the simple ice-flow

Fig. 2 Antarctica, showing the location of the network (short straight line), Byrd Station (BS), and the Amundsen and Ross Seas (A and R).



model is a good approximation for the flow of the ice sheet, supports the concept that the radio-echo layers are connected with the depositional stratigraphy, confirms the conclusion that, at present, this part of the ice sheet is almost in a steady state⁹, and, finally, indicates that this portion of the ice sheet has been close to its present form for a long time: probably >30,000 yr.

The confirmation of the ice-flow model is important. Ice flow at depth can be taken to be in the same direction as the surface velocity; there is important horizontal flow to at least the depth of the deepest recorded radio-echo layer; and the strain rates measured at the surface can be applied through most of the thickness. The model has wide acceptance in the literature but there are few other tests of its validity for an ice sheet. Perhaps the best partial test so far arises from the measurement of the inclination change of the Byrd Station hole²⁶, which showed that the ice velocity vectors under Byrd Station lie close to a single plane, and that there are no sudden changes in shearing rate. This is for just one site, however, and the inclination changes could be measured only for the upper 1,490 m of the ice sheet.

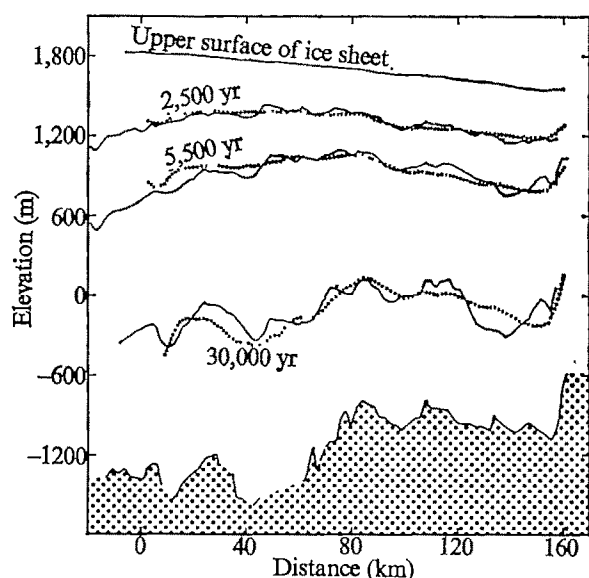


Fig. 3 Profile along the axis of the BSSN. Three internal reflection layers and the base of the ice sheet have been obtained from the radio-echo records. The dotted lines show the calculated positions of ice layers of the ages specified. The ages have been selected so that the isochrons are at the same general depth as the selected radio-reflecting layers.

Analyses of the temperature distribution in ice sheets also find that a steady-state, uniform strain-rate model of flow similar to that used here can describe the measured temperature profiles. Changes in the glacier regime that may have occurred more than several thousand years ago may not, however, be detected by the temperature analyses, and differences in flow direction or vertical strain rate between the upper and lower portions of the ice mass may not greatly affect the agreement of the calculated and the measured temperature profiles.

The success of the model is important to studies that have been conducted with data from the deep core hole at Byrd Station. The ice-flow model used in the analysis of the temperature profile¹¹ and the depth-age relationship for the ice core as calculated by some workers^{17,18} are confirmed. The depth-age relationship obtained from microparticle layering²⁰ is not, however, consistent with the results presented here. Perhaps the microparticle layering is not annual^{17,20}, as was assumed in that work.

The radio-echo layering and the isochrons do not agree exactly. To a large extent this may be because the strain network and the flight lines are in slightly different positions. The depth of the layering is variable in the third dimension, as shown by the results of flights perpendicular to the BSSN axis, and a small navigational error could explain the discrepancies between the radio-echo layering and the isochrons between 0 and 40 km in Fig. 3. Down-glacier from 120 km disagreement is believed to arise from sub-surface advection⁹ associated with the change in flow direction beginning at 140 km, and after 140 km the model calculations are not valid, because the strain network does not follow the flowline. Smaller differences between the model and the radio-echo layering are associated with irregularities in the bed, a factor that is not adequately treated by our simple model.

Each radio-echo layer is probably not caused by a single physical surface within the ice sheet^{21,22}. Sufficiently large changes in permittivity or loss tangent are probably too infrequent to cause all the layering in Fig. 1. More likely, the combined effect of many small changes in permittivity or loss tangent within the ice sheet causes each single layer on the radio-echo record. Each radio-echo layer may be associated with material injected into the atmosphere, perhaps from a volcanic eruption, and the material was for many years seasonally deposited on the ice sheet according to seasonal shifts in weather patterns^{19,22}.

Other explanations for the success of the model?

We suggest that the radio-echo layering is associated with stratigraphic layers of certain ages, but it could also be argued that the radio returns are associated with certain spacings in stratigraphy²¹; for example, annual layering. This spacing would be related to the wavelength of the radio wave in ice, such that reflections from all, or many, of the annual layers would be in phase, and therefore reinforce each other. Using the simple steady-state model for ice flow described above, the depth at which the annual spacing is equal to certain selected values has been calculated and is shown in Fig. 4. The upper two radio-echo layers do not correlate well with these contours and the possibility that only certain annual layer spacings cause the radio reflections is discounted.

Except for the probably small influence of bottom melting or freezing, the oldest isochrons and the thinnest annual layer spacings should both be near the base of the ice sheet, so that very old isochrons and contours of very thin annual layer spacing should be similar, and the fair agreement of the 0.03-m contour in Fig. 4 with the deep radio-echo layer is expected.

It may also be argued that the radio-echo layering is caused by ice foliation²¹ from shearing flow in the ice sheet. This is plausible because bands of different crystal size and crystal orientation are reported for the Byrd Station core²³. Foliation, however, does not become well developed until deeper than 1,250 m below the surface (300 m a.s.l.) at Byrd Station (at 162 km in the Figs)²³ and the radio-echo layering is well developed above this level (Fig. 1). Furthermore, near-horizontal foliation is not likely to have been strongly developed in the ice crest region where horizontal shear strains are orders of magnitude less than elsewhere, yet the radio-echo layering continues, uninterrupted, across the ice crest (at 0 km in Figs 3 and 4). The simplest explanation for the radio-echo layering is that they are isochrons.

Values for surface mass-balances and strain-rates other than those that have been measured could be used to calculate the isochrons. Agreement between the isochrons and the radio-echo layering would be equally good for values of surface mass-balance and strain rate that retain a constant proportion to the measured values (although the calculated ages of the layers at any depth would differ).

There are two distinct surface mass-balance regimes along the BSSN: the first, near the ice crest extending to ~40 km, has a larger surface mass-balance that decreases smoothly with distance from the Amundsen Sea; and the second region, the remaining 120 km, has a smaller surface mass-balance that varies according to the surface slope²⁴. The surface mass-balance is therefore determined by processes acting with different effectiveness in the two regions, so that the climate is unlikely to have changed in such a fashion as to affect both surface mass-balance regions in the same proportion. For example, a change in the frequency of snow-bearing storms from the Amundsen Sea would be expected to affect the surface mass-balance of the ice crest region more than elsewhere along the BSSN. This would change the relative depths of each isochron under the two surface mass-balance regimes and result in a poor correlation with the radio-echo layering.

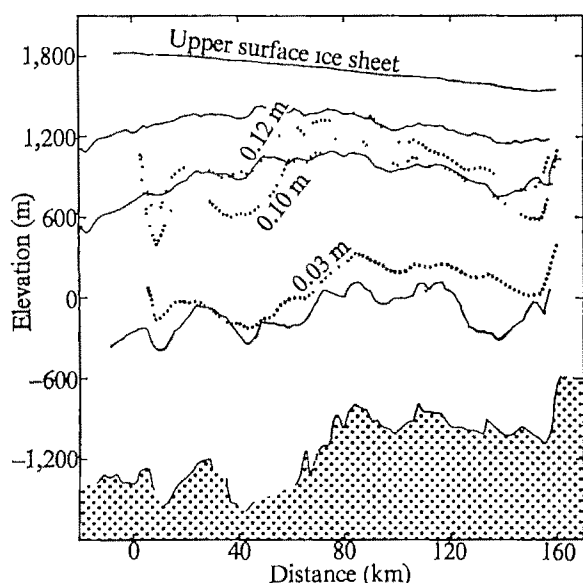


Fig. 4 Profile along the axis of the BSSN. As in Fig. 3, the radio-echo layering is shown by solid lines, but the dotted lines are the calculated positions of ice with the specified annual layer spacing. This is a test of the hypothesis that the observed radio-echo layering may be caused predominantly by the constructive interference of many weak reflections from certain regular spacings in the annual stratigraphy.

A uniform thickening or thinning of the ice sheet with time would change the mean strain rates in a constant proportion, but it is difficult to conceive a mechanism that would thicken or thin the ice sheet uniformly and significantly. Changes in surface mass-balance or air temperature would cool or warm the ice mass at different rates and by different amounts in different parts of the ice sheet, and thus cause important changes in flow lines and dimensions of the ice mass. Similarly, shifts in the position of the ice crest, caused perhaps by changes in the grounding zone, where the ice sheet begins to float in the Amundsen Sea or Ross Sea embayments, would affect the surface mass-balance distribution, and the flow directions and consequently the positions of the isochrons in the ice sheet. Changes at the seaward edge of the ice sheet would affect different drainage basins differently and cause shifts in the position of the ice crest: such a shift would have disturbed the layering of Fig. 3. The radio-echo layering in the BSSN region is consistent with the present-day flow pattern and surface mass-balance distribution and it is very improbable that a major change in flow or precipitation pattern could have resulted in internal layering that so closely follows present-day steady-state isochrons.

Ice sheet stability

The simplest and most plausible explanation for the simi-

larity of the calculated isochrons and the radio-echo layering is that the mass-balance, velocity field, and shape of the central portion of the ice sheet have not changed significantly during the past ~30,000 yr. Small changes in the surface mass-balance or strain rate may not be detected by the technique. The correlation of Fig. 3 is almost equally good using surface data locally changed by as much as ~5% from present-day values.

Other workers have suggested that the West Antarctic ice sheet is behaving unstably²⁴⁻²⁶, but we find that there have been no important changes in the past 30,000 yr. Peripheral parts of the ice sheet could, however, have recently begun retreating rapidly^{27,28}, or have begun advancing rapidly², without the effect having yet reached the region of the ice crest, and we cannot predict future behaviour. The significance of the stability conclusion extends beyond the immediate BSSN region because distant thickness changes would have altered flow directions in the BSSN region and would appear as anomalies in the radio-echo layering. It is difficult to estimate over what area the steady-state result is applicable, but it probably extends 300 km from the BSSN, or over most of Marie Bryd Land.

The technique described here could be applied to other areas where the ice flow can be simply interpreted and where internal radio-reflections can be obtained. It should be used to help select sites for deep drilling and aid in the interpretation of deep core data, and, as shown here, it can be used to determine the balance at present and in the past (since ~30,000 yr BP, in this case) for a large ice sheet.

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Alternating subduction direction and the evolution of the Atlantic Caledonides

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The Morarian, Celtic, Grampian and Lakelandian orogenies are the result of alternating subduction north-westwards and then south-eastwards successively from 1,000 to 450 Myr at the margins of one major ocean basin. Subduction on both margins between 450 and 350 Myr caused the final closure of the basin and the Caledonian orogeny.

THE late Precambrian and lower Palaeozoic history of the north Atlantic region has been difficult to unravel in terms of plate tectonics largely because of the large number of apparent orogenic events and their differing ages in different segments. Most attempts have followed Wilson¹ by starting the history of the Caledonian system with an assumed phase of continental splitting to form the Iapetus² ocean at the beginning of the Cambrian. This separated north-west Scotland, north-west Ireland, and western Newfoundland from England, Wales, south-east Ireland, and E Newfoundland. Such an ocean must have existed at least from Cambrian times, as has been demonstrated³ on palaeontological grounds, but when the late Precambrian history of the region is studied it becomes very difficult to find unequivocal evidence of initial continental rifting. Alkaline activity at 565 Myr in the St Lawrence and Oslo Graben has been suggested⁴ as indicative of the initial rifting of the continental mass. Early Cambrian fossil assemblages from the American and Welsh areas, however, represent highly contrasted benthic faunas which presumably lay on opposite sides of a well developed ocean^{5,6} so it seems unlikely that the time of initial opening was as late as 565 Myr. The most significant feature of the ocean, however, that is difficult to reconcile with a repeated closing and opening, is the independent nature of the plate tectonic events taking place on either margin from 1,000 to 350 Myr.

I suggest here that Iapetus was an ocean of long standing. The successive climactic episodes of orogenic activity (deformation, metamorphism and some types of volcanic and plutonic activity), although nowhere precisely dated, do seem to fall into a remarkably consistent pattern of alternating activity on the north-west and south-east margins. Iapetus presumably had a spreading ridge along its axis, and I suggest that subduction took place alternately to the north-west and south-east, the subduction stopping after a climactic 'orogenic' phase and then transferring to the far side of the ocean. The known pattern of orogenies are thus produced in alternating sequence.

The cause of each orogenic episode then becomes the principal problem. It has recently been recognised that marginal basins may be important in the plate-tectonic evolution of orogenic belts and the Burlingtonian orogeny in Newfoundland has been ascribed⁶ to the closing of a marginal basin. The sequence of events recognised⁷ in the Andean chain of southern Chile seems a likely model. There a cordilleran orogenic belt split behind the subduction zone and a series of marginal basins of oceanic material formed behind a continental sliver. Subsequent closure of those basins was accompanied by considerable compressional

deformation and the subduction in that area probably ceased soon afterwards. The reasons for the marginal basin closure and cessation of subduction have not, so far, been deduced.

A model for the development of the orogenic phases is suggested here (Fig. 1a-d) whereby subduction under a continental edge may lead to the development of a marginal basin beyond the volcanic arc/continental margin which eventually closes and causes an arc/continent collision orogeny. The subduction direction then changes to the far side of the major ocean. This produces a pattern of alternating activity on either margin, which is illustrated for the Caledonian of the North Atlantic by a series of five cartoons which portray the situation immediately preceding each of the following five orogenic events:

- (a) The Morarian⁸ before ~ 1,000 Myr.
- (b) The Celtic⁹ and Ganderian⁸ at ~ 600 Myr.
- (c) The Grampian¹⁰ and Burlingtonian⁶; Tremadoc-Arenig in age.
- (d) The Lakelandian¹⁰ somewhat similar in age to the Taconic of the Appalachians; Upper Ordovician in age.
- (e) The Caledonian or Cymrian¹⁰ orogeny; end Silurian to mid-Devonian from Scandinavia to Newfoundland. This marks the final closure of Iapetus.

The Morarian orogeny

The earliest evidence of continental margin activity on the site of Iapetus comes from north-west Scotland (Fig. 2). The deposition of the Stoer Group¹¹ at ~ 1,000 Myr may mark a post-orogenic molasse deposition to the Morarian orogeny. The Moinian¹¹ lay to the south-east and is a series of trough greywackes, the current markers indicating currents running north-west-south-east. In places it can be seen lying directly on Lewisian basement. It was involved in one or perhaps two periods of deformation¹² and the climax of metamorphism produced a migmatitic core at 1,050 Myr (ref. 13). Later pegmatites were intruded at ~ 730 Myr (ref. 14). The folds include large scale nappes whose direction of transport was probably towards the south-east, and the grade of metamorphism and the ductile deformation of both the crystalline Lewisian and the Moinian sediments suggests that there was a collision orogeny in this region ~ 1,050 Myr ago. This has been termed the Morarian⁸.

A similar event has been recognised in the Ox Mountains inlier of north-west Ireland^{14,16}. The Deer Park Complex of Clare Island has associated amphibolites and serpentinites which continue to the east and north into the Ox Mountains area¹⁶. They are considered to be older than the upper Dalradian and may thus represent part of a Morarian Suture Line. They lie between the possible continuation of the Great Glen Fault and the Highland Boundary Fault—possibly in part even to the south-east of the latter. It thus seems from this evidence that the Morarian metamorphic belt extended south-east of the Great Glen and forms the basement to the Dalradian Supergroup.

It is thus suggested (Fig. 2) that the Moinian developed on the continental margin behind an island arc, and was deposited some way landward of the subduction zone and

any associated igneous activity. The collision of the island arc with the margin of the continent is envisaged as the cause of the Moravian orogeny.

Elsewhere in the North Atlantic region this was the time of the Grenvillian and Riphean orogenies. Both of these areas are characterised by the apparent lack of an extensive depositional episode and the high grade, in places granulite

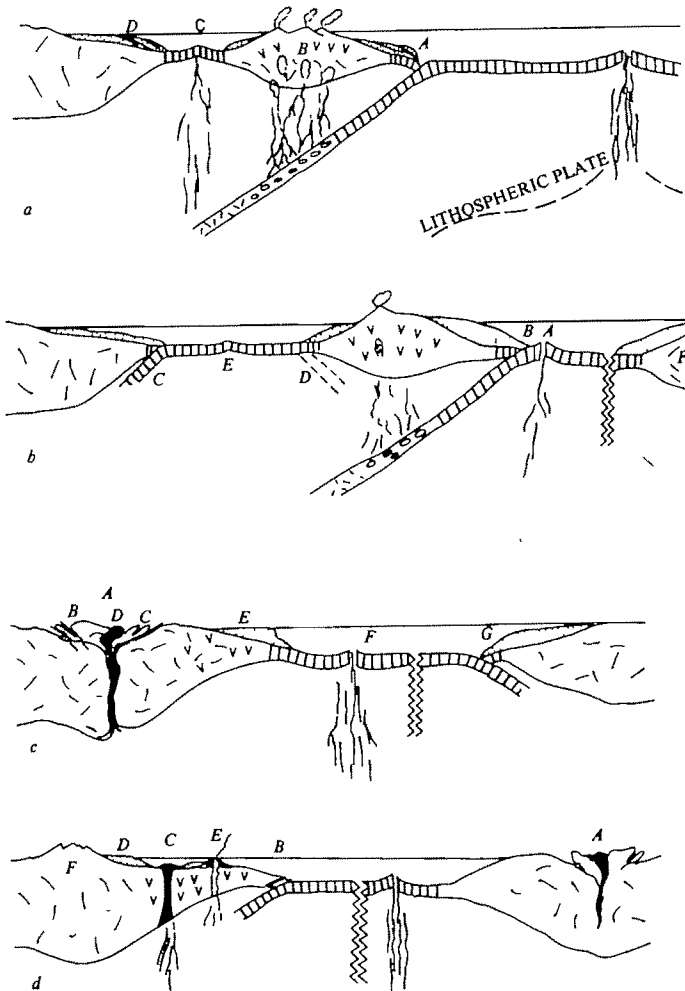


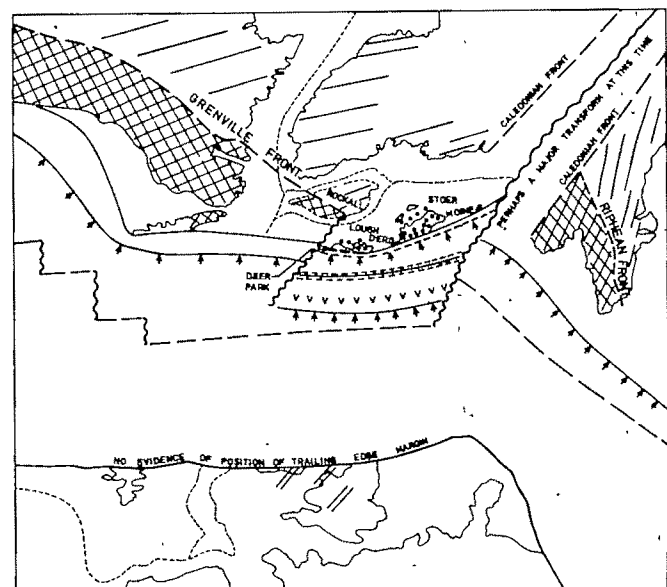
Fig. 1 *a*, Subduction with trench and trough sedimentation (*A*) leads to development of volcanic arc (*B*) either on continental crust thinned by the initial rifting (as shown) or on oceanic crust. Some time after the initiation a marginal basin (*C*) forms behind the volcanic arc and continental margin shelf sediment develops on crust thinned by marginal basin formation (*D*). *b*, Arrival of hot light crust, *A* (mid-ocean ridge) at subduction zone (*B*). This is too light to subduce. Provided that the marginal basin is not too young (that is, its older parts are relatively cool) subduction is initiated within the marginal basin either at *C* or *D*. Sediments at *C* (or *D*) become trough sediments and may be raised to blueschist facies. Cessation of the main subduction causes the marginal basin spreading centre to become a dead ridge (*E*). By this time the far side of the ocean (*F*) has a thick shelf sequence on the trailing edge of the continent. *c*, Subduction within the marginal basin causes collision of the volcanic arc with continent at *A* with nappes on to continent (*B*) and probably on to arc (*C*). Ophiolite (of marginal basin type) and blueschist fragments may be caught in suture zone (*D*). Original trench zone (*E*) now becomes site of shelf sedimentation as this is now a trailing edge, the mid-ocean ridge (*F*) now moving away from this continent toward the subduction zone (*G*) now on the far side of the main ocean. *d*, After the stopping of subduction and orogenesis at *A* (by a sequence similar to *a-c* above) subduction again starts at original side (*B*) and a new marginal basin (*C*) would be initiated in the thinner crust of the original volcanic arc which has been rapidly eroded and covered with shelf sediments (*D*). The new volcanic arc (*E*) may be partly on the older arc or further oceanwards, but is likely to be a good deal nearer the ocean than the original orogenic collision zone (*F*).

facies, metamorphism of early Proterozoic rocks. Anorthosite plutons seem characteristic of this belt and late orogenic granitic bodies as late as 850 Myr occur in Telemark¹⁷ and Sweden. The Grenville front, which probably extends across the Rockall Bank¹⁸, is approximately at right angles to the Riphean front on Bullard, Everett and Smith's reconstruction of the North Atlantic¹⁹. Certainly neither the Grenville nor Riphean fronts represents a suture line, though there may be such within the central parts of these complexes. Thus, although the recent determination of a 'Grenvillian' age for the Moravian high grade metamorphism²⁰ is indicative of orogenic activity at the same time as the Grenville and Riphean events it seems unlikely that the Moravian represents a simple continuation of the Grenville to link it with the Riphean. It may be that south-eastern Scandinavia was at this time on the northerly side of Iapetus (Fig. 2) and that the ocean passed south-eastwards into mid-Europe between Scandinavia and the Low Countries, but evidence is lacking in this critical area. In any case the position of the Moravian seems rather anomalous regarding the amount of sedimentation and the type of deformation. Perhaps the Scottish and Irish areas represented a small back-arc basin to the main Grenvillian-Riphean orogenic belt which elsewhere developed as a Cordilleran orogeny largely by reworking of continental crustal material and without a significant sedimentary prism.

The Celtic-Ganderian orogeny

Subduction then switched to the SE margin of Iapetus (Fig. 3) and probably at this time also the Scandinavian arm of Iapetus was extended NE from Scotland as shown by Precambrian sedimentation in E Greenland through to N Norway. The trough sediments of the Mona and Cullinstown Groups were almost certainly laid down adjacent to, or upon, a continental crustal segment possibly of Archaean age. But they were also near oceanic crust as Anglesey contains most of the indicators of a subduction zone: greywackes intruded by a serpentinite-gabbro diapir, wildflysch, pillow lavas, cherts and basic rocks raised to blueschist facies. The upper part of the sequence contains acid

Fig. 2 Formation of Moravian (> 750 Myr).



- Serpentinites and suture line
- • • Trough Sed's
- Marginal basin spreading centre
- Mid-ocean ridge with transforms
- ▲ Late and Post-orogenic Plutons
- v Volcanics on arc or continent
- Shelf Sed's
- • • Continental Sed's
- Flysch Sed's
- Secondary subduction to close marginal basin
- Subduction zone
- Immediately previous mountain range
- Older crustal remnants

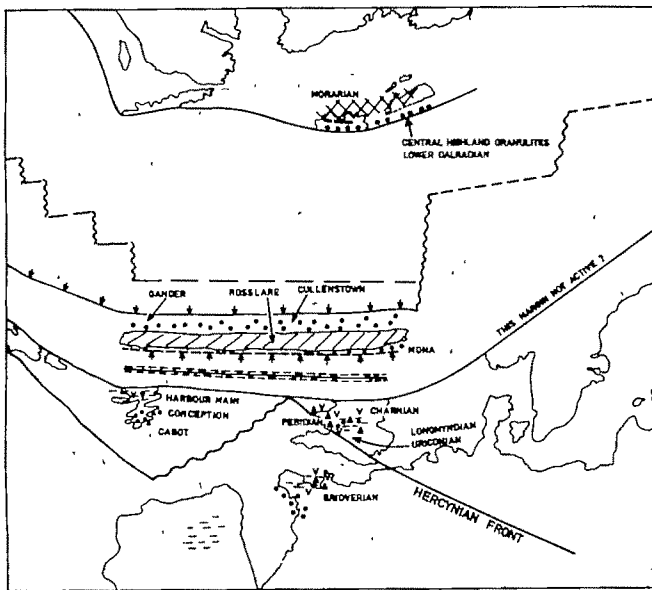


Fig. 3 Formation of Celtic and Ganderian (750-600 Myr). Key as for Fig. 2.

and intermediate volcanics indicating that in the later stages a volcanic arc supplied detritus to the trough area. To the south-east there was a widespread development of calc-alkaline volcanism and plutonism, all probably within the range 750-600 Myr, developed as a fairly typical cordilleran sequence on continental crust.

There seems to be no argument for a longstanding north-west dipping subduction zone since all the contemporary calc-alkaline volcanics lie to the south-east. It is more likely that a marginal basin developed between Anglesey and the Welsh Border and that a collision, closing this basin at ~600 Myr, caused the folding in Anglesey, and brought the blueschists and oceanic crustal slivers to the surface. The evolution of the belt in the English Midlands and Welsh borders follows the normal development of a cordilleran orogeny some way from a continental edge⁸.

In Newfoundland, Kennedy⁶ suggests that a Ganderian orogeny took place on this margin during the latest Precambrian, and advocates the same mechanism, although evidence for a marginal basin is lacking. The Cadomian¹¹ of Brittany and the Channel Isles has been more accurately dated than the rest of this area. It has been suggested²⁰ that Brioverian sediments developed on a south to south-eastward facing continental rise with possibly an ocean indicated by ophiolites in the south. This subsequently became deformed before 620 Myr by east-north-east folding and metamorphism which reached quite high grades in northern Brittany and culminated in the emplacement of late-orogenic intrusives as late as the Lower Cambrian¹¹.

The closely similar timing of the orogenic events of the Cadomian and the Celtic cycles suggest that they were in some way related but their relative positions at this time are unknown. It is possible that the relative positions of these two areas have been markedly altered during the Hercynian orogeny, although Ordovician fossil assemblages point to a single province in the whole of this area to the south-east of Iapetus²¹. On balance it seems most likely that the Brittany area was on a different continental margin. The late Precambrian of north-west Spain which has been compared with the Brioverian was not involved in any orogenic activity at this time²².

The climax of the Celtic orogeny resulted in the formation of a mountainous landmass on the south-east margin of

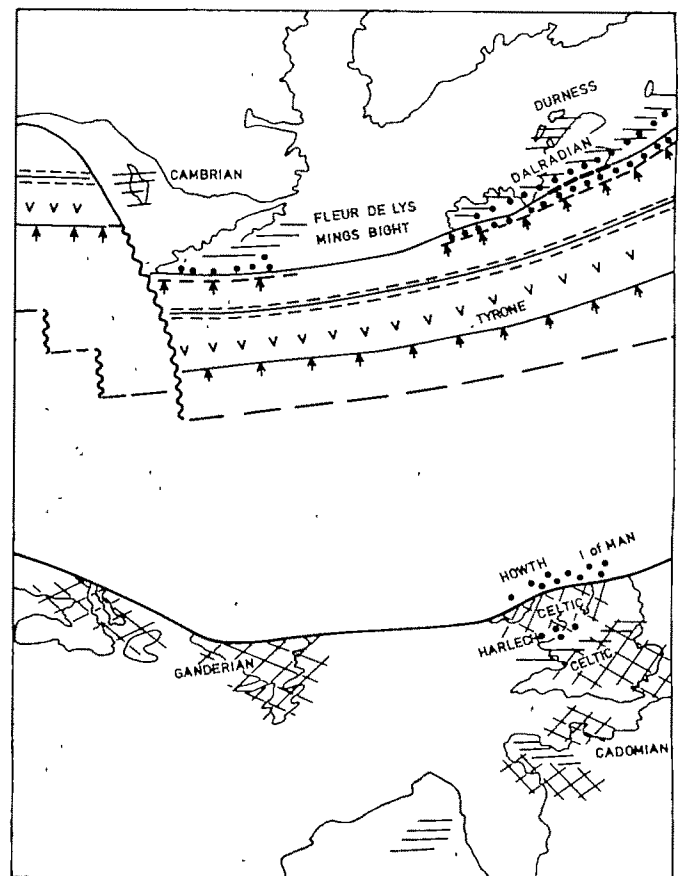
Iapetus and shortly after this there is evidence of increased activity on the north-west side of the ocean.

The Grampian-Burlingtonian orogeny

Following the formation of the Morarian mountains the north-west continental margin continued to receive trough sediments to the south-east of the Great Glen as the Central Highland Granulites (Fig. 4). These sediments show no earlier structural history than that affecting the Dalradian, so must be regarded as the lower part of that sedimentary sequence⁸. Similarly in western Mayo the Erris Group seems to be Moinian type sediment unaffected by any pre-Dalradian deformation¹⁸ and may again represent a post-Morarian early-Grampian trough.

Shelf sedimentation began in the lower Dalradian¹¹ and continued through Middle Dalradian (probably about the end of the Precambrian). All these sediments were being laid down, according to the present model, on the subsiding Morarian volcanic arc. From early Cambrian this shelf extended over on to the Torridonian beyond the Morarian chain, now largely eroded. In upper Dalradian (about Cambrian-Arenig), the continental margin began actively subsiding and great thicknesses of greywackes were deposited, probably marking the beginning of subduction to the north-west again. There are abundant basic volcanics (Tayvallich) in the upper Dalradian but they cannot represent slices of oceanic crust since they lie within a sequence of coarse, shallow-water quartzites. They could, however, mark the opening stage of a marginal basin. The intrusion of the Aberdeenshire layered gabbros (again, not a suture line) apparently after some of the early folding but before the climax of the orogeny indicates proximity to a source of abundant tholeiitic magma, and marginal basin activity would again seem a fairly logical source.

Fig. 4 Formation of Grampian and Burlingtonian. See Fig. 2 for key.



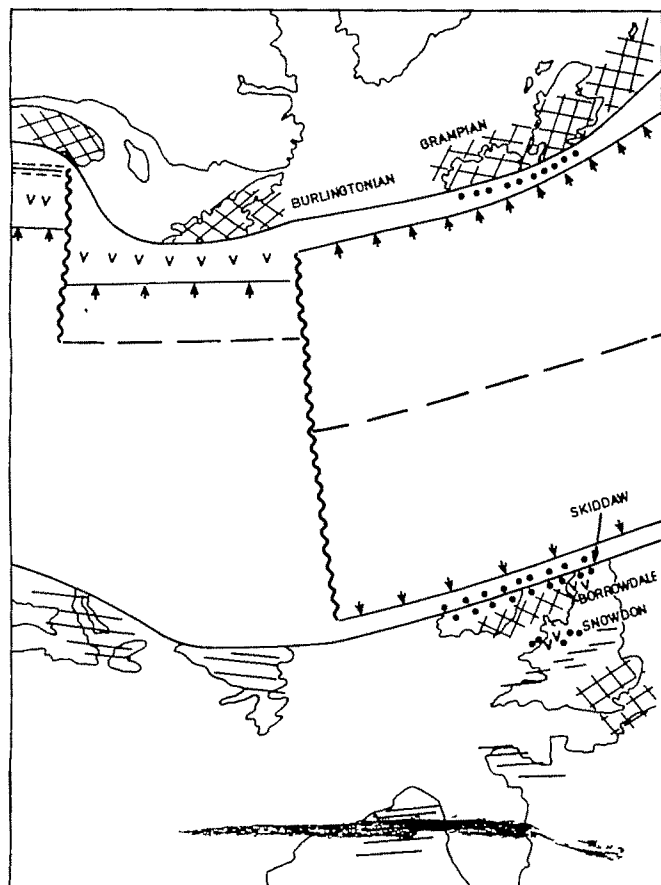


Fig. 5 Formation of Taconic, development of Lakelandian cordillera. See Fig. 2 for key.

The climax of the Grampian orogeny has not been precisely dated but must be about Arenig times. There was intense deformation accompanied by high temperature/high pressure metamorphism, so there is little doubt that it was a collision orogeny. A similar orogeny with clearer evidence of marginal basinal effects has been proposed⁶ for the Burlington peninsula of Newfoundland. This Burlington orogeny took place during the Tremadocian.

The necessity for a substantial collision to cause the Grampian orogeny without closing the whole Iapetus ocean, (and thus involving the Lake District and Wales in a major Arenigian deformation), necessitates the suggested development of an island arc to the south-east of the main part of the Dalradian. This would also explain the lack of calc-alkaline volcanics within the Dalradian sequence, or indeed of volcanics of Cambrian age anywhere in the British Caledonides. The abundant plutonic basic and acid intrusions in the Connemara Dalradian which were intruded at various times in the orogenic cycle could also be indicative of the proximity of that area to the major volcanic arc.

The cryptic suture which does exist along the Highland border (some Arenigian pillow lavas and serpentinite intrusions) probably thus only marks the line of closure of the marginal basin; the major continental fragment which may have had Cambrian calc-alkaline volcanic activity would now lie beneath and south-east of the Midland Valley. The recent LISPB experiment has shown that oceanic crust does not exist beneath either the Southern Uplands or the Midland Valley²³.

The Lakelandian and Taconic orogenies

Following the Grampian orogeny, subduction again switched to the south-east side with the development of middle-upper Ordovician volcanics and trough sediments in the Lake

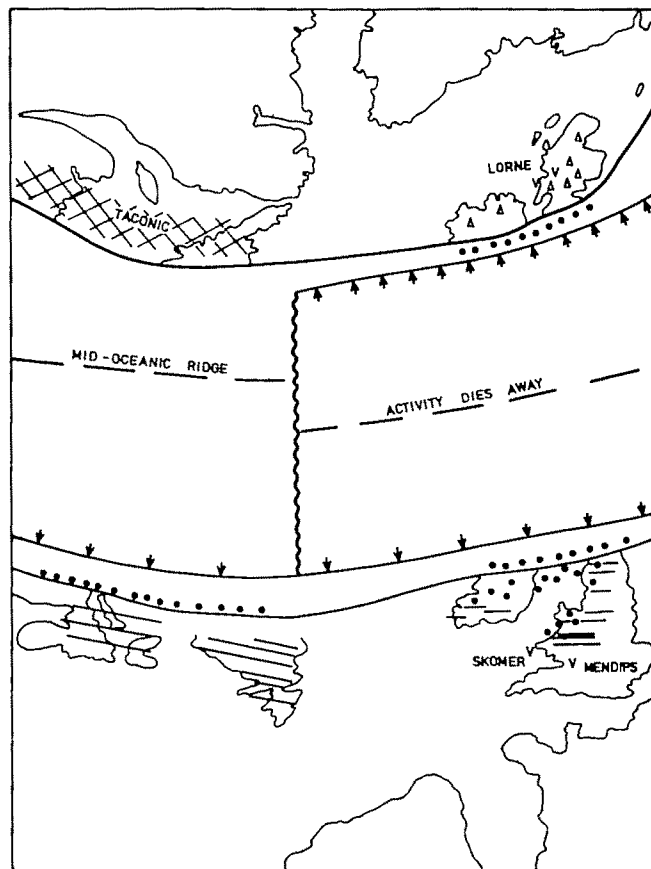
District and Wales (Fig. 5). This followed a period of basinal sedimentation between the Celtic trough area of Anglesey and the Celtic foreland of the Midlands which were both land areas for much of Cambrian and Ordovician times. Although black shales were at times being deposited, the mid-Welsh trough does not seem likely to have been a true oceanic area, but could represent an area of crustal thinning which never quite developed into a marginal basin (for example, the Llanvirn Head Pillow Lavas could mark the opening of such a basin).

There are pre-Borrowdale and pre-Bala earth movements which have been termed the Lakelandian¹⁹ and regarded by some as a major orogenic phase²⁴. It is, however, obviously not a major collision and must be regarded as a cordilleran orogeny on this side of the ocean which never developed a marginal basin or resulted in a major collision orogeny.

On the north-west side of Iapetus (south of the Scottish Highlands) subduction may never have ceased after the Grampian orogeny, since oceanic crust of Arenig to Llandoile age is preserved in the Girvan area²⁵ indicating either oceanic subduction or marginal basin activity in that area very shortly after the Grampian orogeny. In Newfoundland also, tectonic activity in Caradocian times emplaced large slivers of oceanic crust and mantle right over the Burlingtonian core⁶ and indicates that subduction to the north-west continued without much break after the Burlingtonian orogeny in the Newfoundland segment.

Further south-west in the Appalachians, the Taconic orogeny represents a major collision that does not correlate in timing or intensity with the Lakelandian. The age of the Taconic climax of activity is about Caradocian²⁶ and involves major thrusts and nappe type structures which, as with the Grampian, cannot be related to the Ordovician

Fig. 6 Closure of ocean. Formation of Caledonian and Acadian at final collision. Key as in Fig. 2.



history of the south-eastern side of Iapetus. This again implies that the two sides of the ocean were acting independently and the nature of the Taconic orogeny implies a major subduction and collision event in the south-western part of this belt during early and mid-Ordovician times on the north-western margin. It is perhaps most probable that the Burlingtonian orogeny did not take place there but was delayed until Caradocian times in the area south-west of Newfoundland.

The Caledonian orogeny

Whether subduction ceased to the south-east or not (volcanism continued to be active in Silurian times in the Mendips and South Wales), the calc-alkaline volcanism of Lorne and Glencoe suggest that active subduction was taking place beneath the Dalradian, with the subduction zone possibly in the Solway area²⁷ (Fig. 6). It may have been the effect of subduction to both north and south that allowed the closure of the Iapetus ocean, as by the middle of Silurian times the palaeontological evidence indicates that benthic fauna were in contact and by late Silurian or early Devonian the collision had taken place, marine sedimentation had ceased and the rocks of Wales, the Lake District, the Southern Uplands and Central Ireland became quite strongly folded. This is the first time that deformation at approximately the same time can be recognised occurring on both sides of Iapetus, another good indication that this was the first oceanic closure. Along the length of the orogenic belt the intensity of deformation associated with the major oceanic closure increases from Newfoundland (where it was mid-Devonian and very mild) to Scandinavia where it was much more intense than in Britain. In Scandinavia the Caledonian orogeny was so intense that Silurian rocks are metamorphosed and involved in major thrusting, so that the correlation of the orogenic events in that segment with the sequence proposed here has not been attempted.

This sequence of orogenic climatic events is thus envisaged not as a continuous orogenic cycle operating along a continental collision-site as many previous syntheses have portrayed the Caledonides, but as a discontinuous series of subduction/marginal basin formation/closure-collision events on either side of a long-standing major ocean which only finally closed in Silurian to mid-Devonian times and thus stopped any subduction or ocean spreading in this region. The model is clearly susceptible to testing by palaeomagnetic determination and may need revision in the light of local stratigraphic detail. The model does, however,

draw attention to one of the major features of plate tectonics applied to older palaeogeographic reconstructions that has not been too clearly recognised by many writers, namely the independence of events on one side of an ocean from contemporaneous events on the other. The correlations between the sedimentary history of the Mona complex with possibly contemporaneous sediments in the Dalradian or Brivoerian will be practically impossible, and largely irrelevant, if these three areas were on three different continental margins at different stages of activity or of different geotectonic type. Similarly, the search for the equivalents of the Taconic orogeny in southern Britain are irrelevant if the two areas were on margins of two different, independently acting, continents.

A major problem now apparent from the series of suggested events (Figs 2-6) is the reason for the closure of Iapetus and specifically why its mid-oceanic ridge stopped spreading. One must look again to a coupled series of events and the beginning of the Hercynian orogeny is closely related to the end of the Caledonian, so that it seems very likely that the starting of a major site of crustal extension and the formation of an ocean on the margins of which the Hercynian orogenies developed²⁸, was the cause of the failure of the Iapetus spreading centre and ultimately the closure of that ocean forever.

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letters to nature

Oblateness of the atmosphere of Mars

ON April 8, 1976 Mars occulted the bright star α Geminorum (magnitude 3.2). Predictions¹ were issued well in advance since such occurrences are very rare: indeed, it is estimated that Mars occults a star as bright as this about once in 500 yr. Accurate observations of the duration of the occultation were made from four sites on the Earth and these are analysed here for the shape of the Martian atmosphere at that height at which the intensity of the starlight diminished by half. The oblateness is found to be about twice as great as that of the surface of Mars.

The occultation was widely observed throughout North America, the Caribbean area and Great Britain, mainly visually, although a number of photoelectric observations were also made. Of particular interest are the high accuracy photoelectric observations made from Greenbelt (Maryland), Atlanta (Georgia), Fort Davis (Texas) (G. de Vaucouleurs, et al., to be published) and from an aircraft over the western North Atlantic². Timed observations of '0.5 light' at the disappearance and reappearance phases of the occultation have been reported from these four places. The aircraft observations, from a height of > 12 km, were made at 4,500 Å—a shorter wavelength than that used by the ground

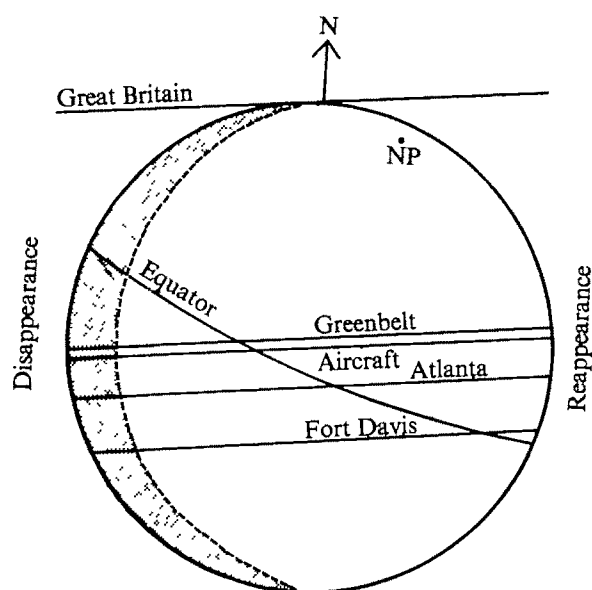


Fig. 1 Occultation of ϵ Geminorum by Mars on April 8, 1976 as seen by various stations. NP marks the North pole. Although the chords representing the photoelectric observations are nearly central, there is a good spread in latitude because of the tilt of the axis of Mars.

observers. Because of the very low altitude of Mars it was not possible to make photoelectric observations from the British Isles. This was unfortunate since a grazing occultation occurred from southern England, Wales and Ireland, and observations from localities such as these would have improved the coverage of the Martian disk considerably. This is clear from Fig. 1.

Using the known distance of Mars and its direction and angular rate of motion, it was possible to calculate the apparent positions of the star at the observed times, relative to the centre of Mars. A standard computer program, written by the author, for the analysis of previous occultations of stars by planets and satellites was used to solve for three unknowns—two for the position of Mars and one for the correction to the adopted equatorial radius. Several solutions were found using various trial values for the flattening of the Martian atmosphere, where the flattening is defined as [(equatorial radius—polar radius)/equatorial radius]. Calculations were also done without the aircraft observations since these were made at a shorter wavelength than the others and thus refer to a greater height in the Martian atmosphere. This did, indeed, prove to be the case, the height being ~ 6 km above the ellipsoid fitted to the other observations.

The position of the star was taken from the FK4 catalogue, while the ephemeris of Mars was obtained from the Jet Propulsion Laboratory. The analysis of the ground-based photoelectric observations showed that this ephemeris was extremely accurate. In fact, the derived correction to the ephemeris of Mars relative to the star was only

$$+0.0019 \pm 0.0001 \text{ s in right ascension (RA)} \\ +0''.009 \pm 0''.007 \text{ in declination (dec.)}$$

This correction is considerably less than the s.e. in the position of the star itself.

For the first solution the adopted value of the flattening was 0.0052, which is the dynamical flattening given for the surface of Mars by Lorell¹. By varying this value, however, it was quickly found that the s.e. of all three unknowns decreased as the flattening was increased and that the sums of the squares of the residuals went through a minimum when the flattening was ~ 0.012 – 0.014 . The results are

Table 1 Computer fits

Adopted flattening	Equatorial radius (km)	RA (s)	Correction to dec. (")
0.0052	$3,458.4 \pm 3.6$	$+0.00173 \pm 21$	$+0.0145 \pm 135$
0.0097	$3,461.6 \pm 2.3$	$+0.00185 \pm 13$	$+0.0115 \pm 87$
0.0135	$3,464.4 \pm 1.9$	$+0.00195 \pm 11$	$+0.0089 \pm 71$

given in Table 1. The residuals of the observations were very good—the greatest residual in the radius being 3.9 km, which corresponds to a timing residual of only 0.18 s.

The values for the equatorial radius have not been corrected for refraction. To obtain the true distance from the centre of Mars the scale height should be added. This is probably ~ 10 km, but will doubtless be determined more accurately from the individual light curves at a later date: it should then be possible to improve the solution by applying the scale height to each observation individually.

Table 2 Flattening and equatorial radii at various levels

Level	Flattening	Equatorial radius (km)	Method
Surface	0.0057 ± 0.002	$3,394 \pm 4.5$	Spacecraft radio occultation ⁴
?	0.0091 ± 0.002	$3,402 \pm 16$	Visual micrometer observations ⁵
0.5 light	0.013	$3,474 \pm 3^*$	Photoelectric occultation

*Includes an estimated error for the scale height.

Table 2 shows the flattening and equatorial radii at various levels. For comparison purposes the radius derived from the photoelectric observations has been increased by an assumed scale height of 10 km.

It may eventually be possible to express scale height as a function of latitude. Since the observations at each phase range over only 25° in latitude, however, it is unlikely that there will be a marked change in the flattening.

The cause of this observed flattening may partly arise from a diurnal variation in density—the disappearance phase occurred on the unilluminated limb while the reappearance phase occurred on the illuminated limb. No such effect, was, however, detected when Jupiter occulted a star in 1971, the flattening at 0.5 light level being very close to the dynamical flattening⁶.

In conclusion, it would seem from this analysis that the 0.5 light level has an equatorial radius of $3,464 \pm 2$ km (+ scale height) and a flattening in the range 0.012 to 0.014.

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Hydrodynamics and geodynamics in the Phlegraean fields area of Italy

WE show here, from an analysis of geological observations in the Phlegraean Fields, that the high correlation among slow ground motions, seismicity and short and long period tidal components (including the Chandler wobble) observed at Pozzuoli (Naples) suggests that the sea, throughout intercrustal

hydrodynamic correlation, may be an important geodynamic agent.

The intense Phlegraean–Vesuvian volcanic event occurred in Campania (southern Italy) in the area lying between Monte Massico, the margins of the pre-Apennine arc, the Sorrento peninsula and the Gulfs of Naples and Pozzuoli¹. The Phlegraean Fields are the volcanic district W of Naples (Fig. 1a), some of whose craters form part of the city's urban system. The Phlegraean volcanic activity, as opposed to that of Vesuvius which is famous for its lava manifestations, is mainly characterised by huge explosions of the phreatic type, with ejection of predominantly ash products. A thick buildup of which is followed by intense diagenesis with the formation of the classic Phlegraean lithoid tuffs. The characteristics of these tuffs are their considerable cohesion and very high porosity (40% at the surface); consequently their rheological behaviour can be compared with that of a sponge dipped in water².

The morphology of the Phlegraean Fields is characterised by shallow craters sometimes affected by marine erosion, by many thermal springs both on land and under water, some feeding the

Fig. 1 Phlegraean Fields volcanic basin. a, First-order residual gravity anomalies. Also shown: survey points; morphological and structural borders of the caldera; the west-east and north-south trends of the profiles of Fig. 1b and Fig. 4 respectively. b, Profiles of the maximum uplift (—▲—▲—) and residual gravity anomalies (—), with the geological cross section according to the west-east trend.

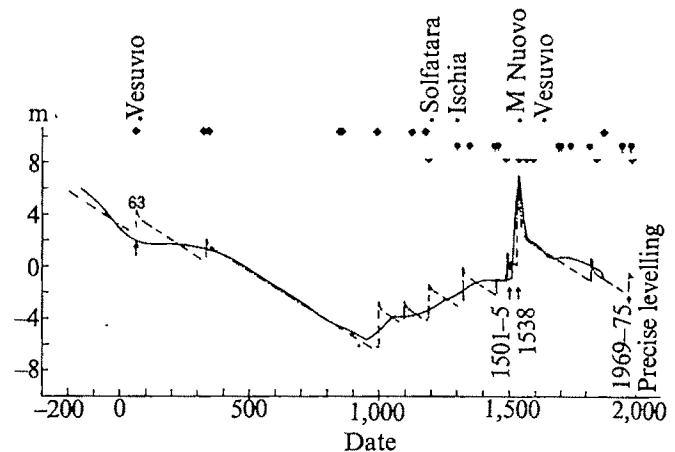
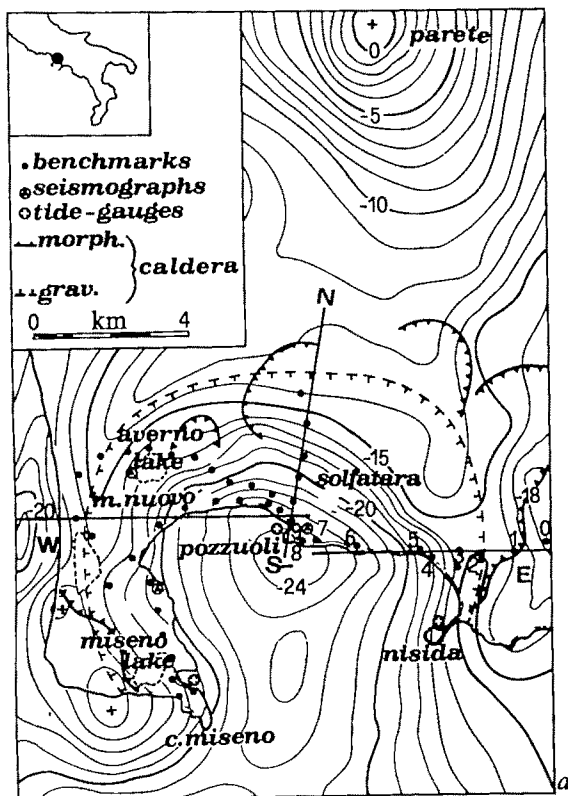
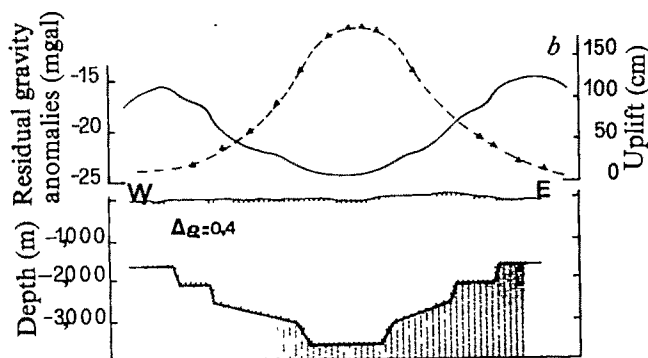


Fig. 2 Trend of Phlegraean bradyseism over the centuries. (—) Parascandola's trend; (— · — · —) represents the modified curve. ↑ gives date of historical uplift; *, a major eruption in Campania; ◆, a major earthquake in Campania; ●, a major earthquake in Campania, also causing damage in Naples; ▼, an earthquake in Pozzuoli.

spas that were already famous in Roman times, and by solfatara fields. The dynamics of the Phlegraean Fields is characterised by a vertical movement of the crust to which has been attributed the Greek term 'bradyseism'. The latter brought about one of the longest series of continuous up-and-down movements. In fact, the trend of this local vertical movement, that has continued for many centuries at a time, has been reconstructed from the study of the clear traces left by the lithodomes on the surface of the columns of the Roman *Mercatum*, called Serapeo, built in the second century BC at Pozzuoli.

Figure 2 shows the diagram drawn, as an unbroken line, by Parascandola³, which is also based on the data collected by Nicolini⁴ in the middle of the nineteenth century. It is particularly interesting to note that in 1538 there was a ground uplift of ~ 7 m in only 48 h when the eruption of Monte Nuovo took place, and that the maximum uplift was recorded in Pozzuoli at ~ 3 km from the point of eruption.

In the study of the origin of Phlegraean bradyseism⁵ the lowering phenomenon is interpreted as being a result of compaction through overloading of the light pyroclastic products filling the Phlegraean basin down to a depth of 3.5 km (gravimetric model shown in Fig. 1b).

The uplift, on the other hand, is interpreted as being the result of the rheological behaviour of the porous fluid-saturated media affected by the flow variation in these same fluids as a consequence of both a variation in the amount of heat carried up from below (increase in the convective movements) and the seismoeruptive effects of the phreatic explosion.

Moreover, as it is well known that earthquakes are also accompanied by ground uplift events, which, although much smaller than those recorded for the Phlegraean area, have similar characteristics, Parascandola's diagram has been suitably modified as shown in Fig. 2 with the dash and dot line.

The proposal to modify the trend of the historical data was made on the assumption that when the local and regional eruptions and earthquakes take place there may also be discontinuity, with a rapid ground uplift and a successive return to subsidence conditions. Only the eruptions of Vesuvius of the years AD 79 and 1631 have been considered, as they began new eruptive cycles after a few centuries of inaction.

The hypothesis that the Phlegraean uplift movements take place over short periods of time is confirmed by the recent inversion of bradyseism noted in early February 1970, whose development is shown in Fig. 2.

The recent event, which probably began in 1969 and continued for four years, has enabled the study of some of the characteristic manifestations.

The inversion was seen to be accompanied by a continual increase in steam flow from the fumaroles and a widening of

Solfatara di Pozzuoli mud pool. The increase and decrease of these reflected the ground uplift trend. No variation in the temperature of these same manifestations was ever observed.

The data we have analysed were gathered by several companies and organisations⁶ and covered the following: ground movement, microseismicity and sea movement.

In the case of the ground uplift that took place from March 1970 to February 1973 the Campanian Public Works Office carried out precision level measurements on several tens of benchmarks throughout the Phlegraean zone. The heights had been checked various times between 1903 and 1953. Along the Naples (Fuorigrotta)–Pozzuoli coastline the measurements made on benchmarks numbers 0–9 were made on average at monthly intervals. The resulting uplift data were found to have both area (Fig. 1b) and time dependencies (Fig. 3). Figure 3a shows that the ground movement trends with respect to the maximum height reached in the individual benchmarks are in close agreement with one another.

The maximum uplift was reached in July 1972 (Fig. 3) and the average trend for benchmark 7 (where the absolute maximum was reached) can be expressed by a typical diffusion equation

$$q = q_0 + m \exp[-\lambda_1(t_0 + t)] - n \exp[-\lambda_2(t_0 + t)]$$

where q is the height reached, m , n , λ_1 and λ_2 are constants

Fig. 3 Time dependence of the ground uplift in the Phlegraean Fields. *a*, Variation (with respect to March 9, 1970) in the height of the benchmarks assuming for each one the height reached on July 6, 1972 (referred to as q_m in the figure) to be 1.0. Benchmark 0 is regarded as being stable. This shows the average value for each series of measurements and the interval (I) containing the random distribution of the single values from the 9 benchmarks. For benchmark 0, q_m was 0 (cm); for 1, 3.00 cm; for 2, 8.5; for 3, 17.36; for 4, 28.75; for 5, 35.89; for 6, 69.51; for 7, 70.37; for 8, 68.46; for 9, 64.60. *b*, High accuracy levelling values for benchmark 7. Ordinates (origin at the level of May 19, 1969) are the lower set on the left and go on near the curve. *c*, Best fit curve (see text); *d*, *e*, *f*, trends obtained from tidal data given in Corrado and Palumbo⁶. Ordinates are on the right (the origin is not at the same level as the *b* and *c* curves).

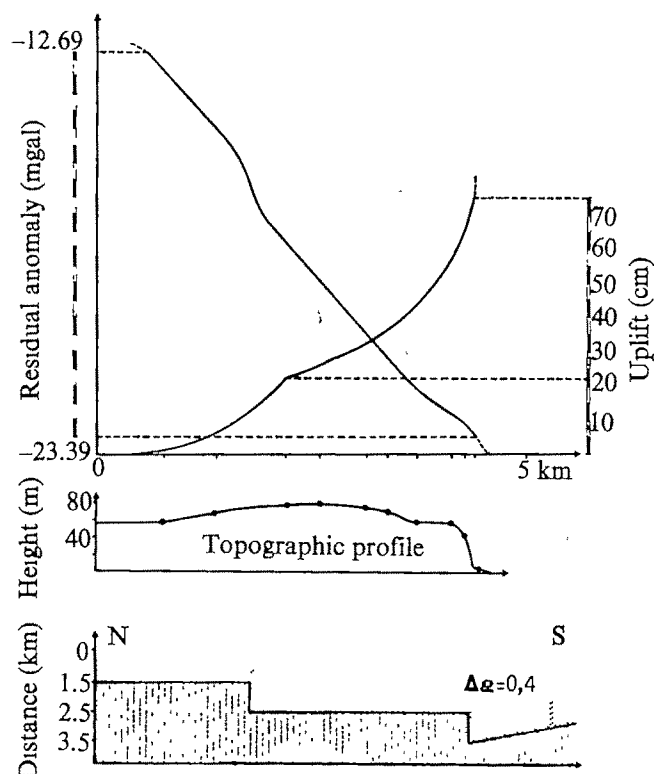
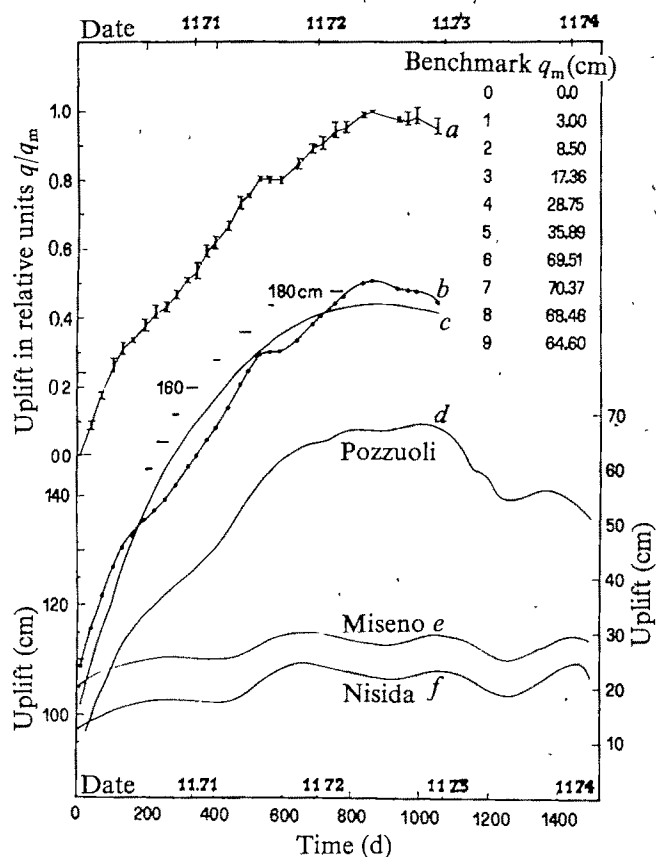


Fig. 4 Trend of uplift from 1962 to 1970 and (n-1) the order gravimetric residual anomalies; topographic profile and geological cross-section along the N-S profile indicated in Fig. 1a (after Montagna⁷).

chosen to give the best fit to the experimental data; t_0 is when the bradyseism inversion began (\sim May 19, 1969; $t_0 = 285$ d) when $q = -23.77$ cm, compared with the height measured in 1953 by I.G.M. This is in agreement with the dependence of uplift on the variation of the flow of fluid within porous layers (Fig. 3c).

The uplift profile (Fig. 1b) shows an increase from the borders of the caldera inwards, that is, from almost negligible values to 156 cm compared with the height in 1953 (180 cm compared with May 1969 when the inversion of the phenomenon probably began). The uplift values can be connected with different thicknesses of the pyroclastic layer (Fig. 1b).

During the recent uplift, the altimetric survey made by the Italian Geological Survey in 1962 (ref. 6) along the N-S profile of Fig. 1a was repeated (April 1970) and a more detailed gravimetric survey⁷ was also carried out. The two-dimensional model, drawn from the first-order residual anomalies, compared with the height variations, shows a clear correlation between the type of uplift and the thickness of the pyroclastic products covering the limestone substratum. Figure 4 reveals the correspondence between the uplift discontinuities and the position of the faults⁷.

These observations are in agreement with the data on the ground movement obtained⁸ by comparing the average sea level far away (the Naples and Pozzuoli tide-gauging stations).

The correlation coefficient between the data in Fig. 3b and those in Fig. 3d was found, by Pearson's formula of the product moment, to be 0.994.

The curves shown in Figs 3 and 5c reveal that the uplift in the different stations in the Phlegraean Fields between 1970 and 1975 has a trend which is the combination of different periodical components.

Three seismic stations have been in operation⁸ since March 1970 to study the seismicity in the Phlegraean Fields. At the Pozzuoli station (Vescovado: Fig. 1a), where the majority of the seisms have been recorded, 5,752 seismic events were noted up to February 1974. The foci were essentially concentrated within a depth of 3.5 km and had a random distribution.

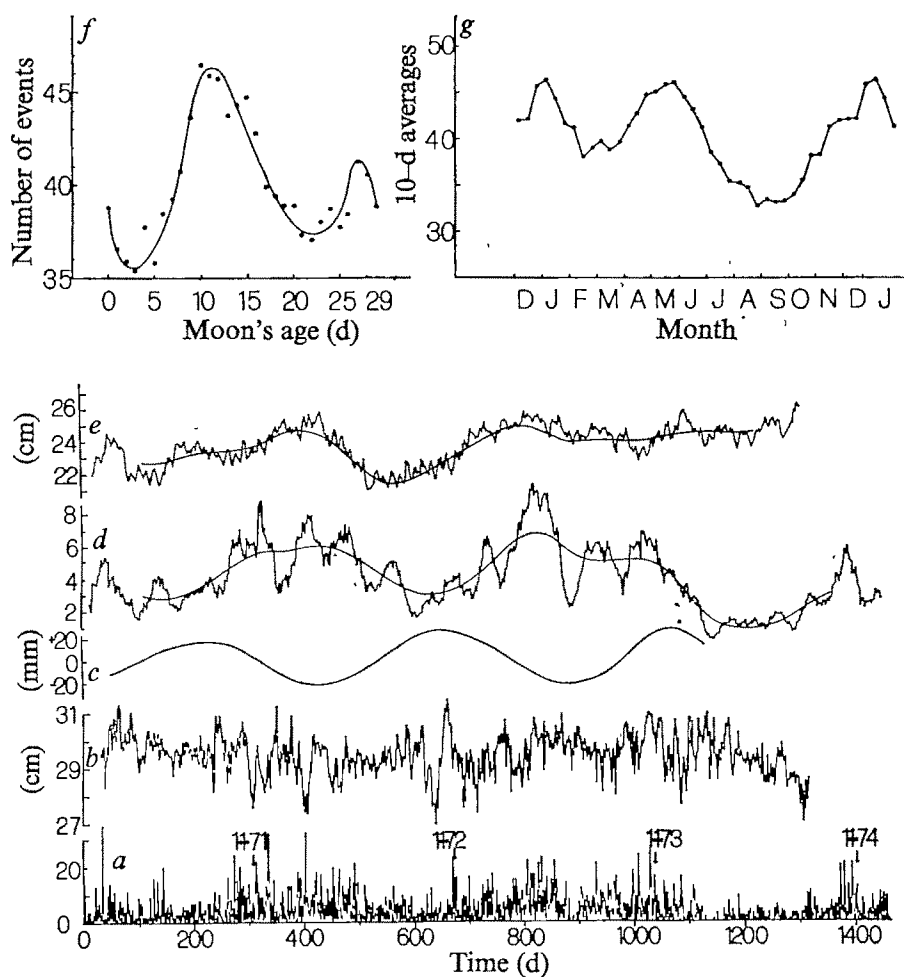


Fig. 5 Trend of seismicity, vertical ground movement and sea movement at Pozzuoli. *a*, Number of daily seismic events recorded at Vescovado station; *b*, Average daily sea level; *c*, Ground uplift calculated by subtracting the average trend values from those of the curve in Fig. 3*d*; *d*, average daily number of seismic events for 29 consecutive days along with the relative average trend; *e*, analogous values and average curve for the tide amplitude; *f*, average daily seisms in function of the Moon's age for the period 1970-74; *g*, average 10-d seisms for the same period

Only about ten of these were felt by the local population, having reached an intensity of III-IV Mercalli degrees.

Also the daily trend of the seisms is clearly periodical (Fig. 5*a* and 5*d*): the long period trend is accompanied by several short period trends, in particular the fortnightly (Fig. 5*f*) and six-monthly (Fig. 5*g*).

The average daily sea level (Fig. 5*b*) and the sea-level variation over 6 h (an index of its mean velocity), obtained by subtracting the low tide level from the previous high tide level (Fig. 5*e*), refer to Pozzuoli station (Capitaneria di Porto). The latter curve also shows, besides the smaller period trends, a clear long period one.

The correlation coefficient of the average curve drawn from Fig. 5*d* and *e* is 0.81, also calculated from Pearson's formula: the 99% confidence limits are 0.69 and 0.89.

This suggests that there is a direct connection between the sea motion, considered as a 'tidal pumping', and the micro-fracturing of the skeleton of the highly porous and permeable pyroclastic layer.

The trend of the uplift curve (Fig. 5*c*), obtained at Pozzuoli by subtracting the average uplift values from the values of the curve in Fig. 3*d*, is analogous to those of Fig. 5*d* (daily seismic events) and 5*e* (tide amplitude) and the correlation coefficient, as calculated once again from Pearson's formula, between values in Fig. 5*c* and *d* is -0.73: the 99% confidence limits are -0.84 and -0.57.

In spite of the short interval considered (1,400 d) the fact that the three phenomena have a common period of 400-450 d brings to mind the Chandler wobble. To confirm this connection the analysis of tidal and geodynamic (earthquake and vertical ground movement) data should cover a period of at least 7 yr.

In conclusion, it would seem that the phenomena accompanying the Phlegraean bradyseism give support to the interpretation of its genesis² based on the alteration of intercrustal

hydrodynamics, and in particular they suggest that the sea has an influence on local geodynamics.

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Ophiolite emplacement on to continental margins

RECENT work on west Newfoundland ophiolites supports Elliott's¹ contention that the mechanics of emplacement of oceanic crust and upper mantle on to continental margins ('obduction') is no different from the emplacement of thrust sheets in any foreland thrust belt. Evidence to this effect comes from the presence of minor structures found along the thrust fault surfaces separating the various structural slices, as well as from other related minor structures—hitherto unrecorded in ophiolite suites—that have been found within the thrust sheets themselves. These

reveal a very specific kind of information about the mechanics of ophiolite transportation and, without reference to the forces causing emplacement, they imply that many of the processes involved in the motion of ophiolite thrust sheets are equivalent to those typical of foreland thrust belts—due consideration being given to basic differences in the material properties of the rocks involved.

As far as the basal metamorphic aureole associated with the western Newfoundland ophiolites is concerned, it is clear that it predates their transportation on to the continental margin. Direct evidence to this effect is found in the Bay of Islands complex² where the low greenschist grade thrust faults that are responsible for ophiolite transportation obviously truncate the higher grade aureole, placing it and the adjacent ophiolite stratigraphy structurally over and above the unmetamorphosed oceanic sedimentary rocks. In other places this low greenschist grade structural base may follow the pre-existing metamorphic aureole³, using it as a suitable discontinuity, analogous to a bedding plane thrust in a foreland thrust belt; although this would appear to obscure the history and chronology of the two events (that is, an earlier one that produced the higher grade metamorphic aureole, from a later one that emplaced the ophiolites on to the continental margin along greenschist grade thrust faults), a distinction can be made on the basis of minor structures observed within the thrust sheets themselves. These take the form of narrow ductile deformation zones (DDZ, see Fig. 1) formed during the motion of the ophiolite sheet; locally these clearly truncate and retrograde to greenschist facies the granulites and amphibolites of the basal aureole, as well as parts of the overlying ophiolite stratigraphy. Such relations express, rather explicitly, the lack of significance of the basal metamorphic aureole in terms of the immediate processes inherent to the orogenic belt in which the ophiolites have been emplaced. In terms of its pre-emplacement significance, one wonders if the high temperature aureole might be explained as the product of dynamic conditions induced during seafloor spreading, by the lateral flowage of oceanic crust and upper mantle in the vicinity of an ocean ridge? Or, could it be the vestige of a subduction zone fabric?

With respect to greenschist grade emplacement mechanics, the presence of narrow ductile deformation zones throughout a large volume of an ophiolite thrust sheet shows that there is a fundamental difference in the internal energy dissipation⁴ within an ophiolite thrust sheet, compared to that of a foreland thrust sheet involving only unmetamorphosed cover rocks. In the latter, a large proportion of energy is absorbed by folding, generally taking advantage of the facilitating slip planes provided by well developed bedding. In contrast, in the massive or crudely layered crystalline rocks which constitute most of an ophiolite sheet, deformation is much less homogeneous, taking place along narrow, often distantly spaced, ductile deformation zones. Their number and intensity varies according to position within the ophiolite thrust sheet; although no attempt has yet been made to quantitatively evaluate this, clearly they are much more widely spaced and weakly developed throughout the bulk volume of the thrust sheet than near its base. In the proximity of the fault plane (within 30 m) there is a seemingly exponential increase in the number of DDZ; at the fault, the deformation zones run together, with small ones feeding larger ones—in effect, emptying their displacement cumulatively into large shear zones which subsequently empty theirs into the main thrust fault at the base of the ophiolite slice. Similar ductile deformation zones have been described by Ramsay and Graham⁵, and Mitra (unpublished) in deformed crystalline basement and intrusive rocks of several orogenic belts. Their significance here, in terms of ophiolites, lies in their recognition for the first

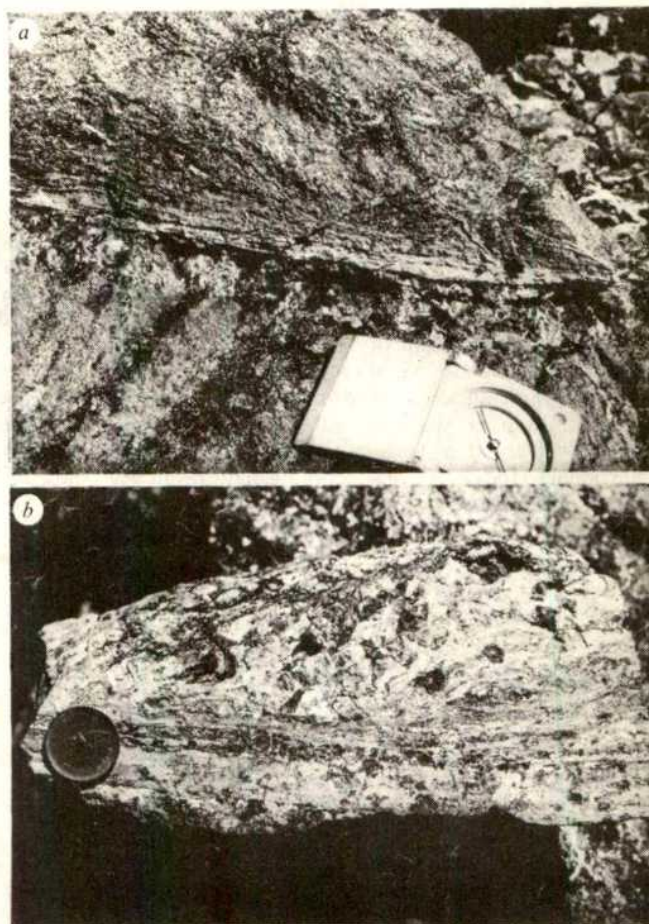


Fig. 1 *a*, Asymmetric ductile deformation zone cutting across the gabbro cumulates, east side of Blow Me Down Mountain, Bay of Islands complex. *b*, Close-up of a sample from the same deformation zone, showing the spectacular grain-size reduction of the original mineralogy (coarse pyroxenes in feldspar matrix) to a narrow, greenschist-grade mylonite.

time as diagnostic minor features related to the transportation and emplacement of oceanic crust and mantle on to a continental margin. Although in terms of internal energy dissipation they record a fundamental difference between ophiolite and foreland thrusts, this is only important in consideration of the material properties inherent to the specific thrust sheet.

Information on the physical mechanisms operative at the base of a moving ophiolite thrust has been recorded by a variety of minor structures along the actual fault surface. These include: (1) fibrous accretion steps (micro-lite fibres where ultramafics occur in the hanging wall), and (2) striations, gouge, and polished surfaces, similar to those features described by Elliott. These are indicative of pressure solution slip and frictional sliding processes operating at the base of a moving ophiolite sheet, as in the case of a foreland thrust⁴.

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Water movement in porous media towards an ice front

IN frozen soils water movement towards an ice front can occur, leading to the formation of ice lenses, frost heaving and damage to roads and other structures. During the past decades many attempts have been made to explain the transport phenomena involved, fundamental contributions being made by Everett¹ and Takagi², among others. We present here experimental results on the influence of pressure on ice growth; they accord with thermodynamic theory.

At our laboratory Vignes and Dijkema³ studied the flow of water towards an ice front through a very narrow slit ($50\text{ nm} \times 1\text{ mm} \times 50\text{ mm}$), all walls consisting of quartz. They found that under isothermal conditions the flow occurred at a rate proportional to the degree of subcooling. This result was confirmed at our laboratory by De Loos (internal report) who studied flow of water towards an ice front through a glass cylinder of 1 mm^2 cross section, containing 10^4 parallel cylindrical capillary channels of $1\text{ }\mu\text{m}$ diameter.

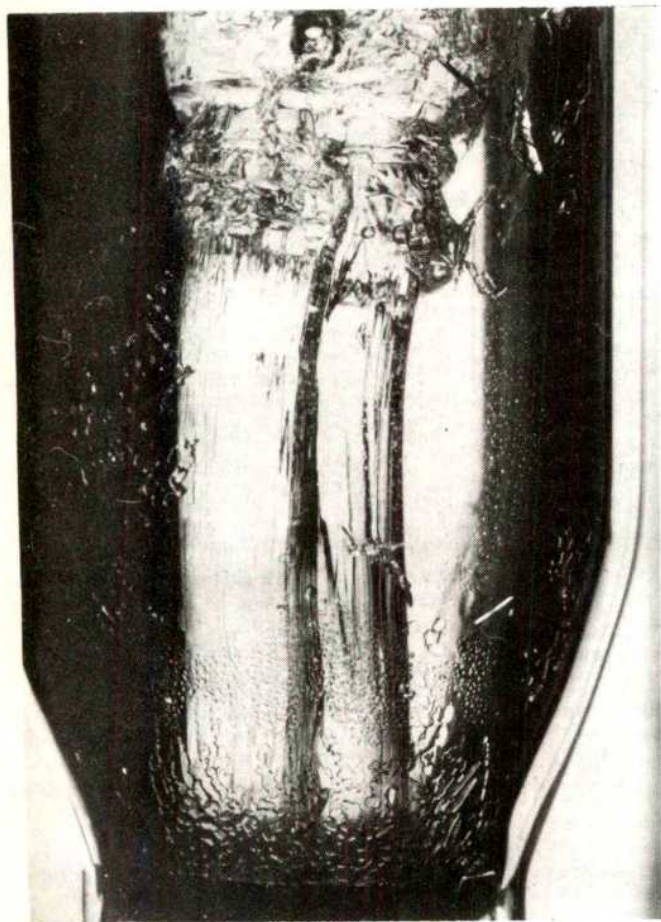


Fig. 1 Ice column (diameter $\approx 7\text{ mm}$) formed during 15 d after starting ice growth. $T = -0.045^\circ\text{C}$, $p_i = p_w = 101\text{ kPa}$.

Vignes and Dijkema³ explained their experimental results by suggesting that the seat of the phenomenon is to be found in the thin layer of unfrozen adsorbed water between the ice and the quartz wall. The pressure distribution in this layer is thought to be anisotropic, leading to heaving of the ice by the disjoining pressure on the one hand, and water flow by suction in a direction parallel to the wall on the other hand. This was

elaborated on by Vignes⁴ in a theoretical study, treating the thin water layer as a micropolar fluid.

In subsequent experiments we extended this work by investigating the influence of pressure on the rate of water movement. Ice and super-cooled water were separated by a horizontal glass filter, all walls consisting of glass as well. The ice was situated on top of the filter. The pressure of the water and the ice could be controlled independently.

Under isothermal conditions, and at atmospheric pressure on both ice and water, water flow towards the ice was again observed. Figure 1 shows an ice column formed during 15 d of stationary conditions.

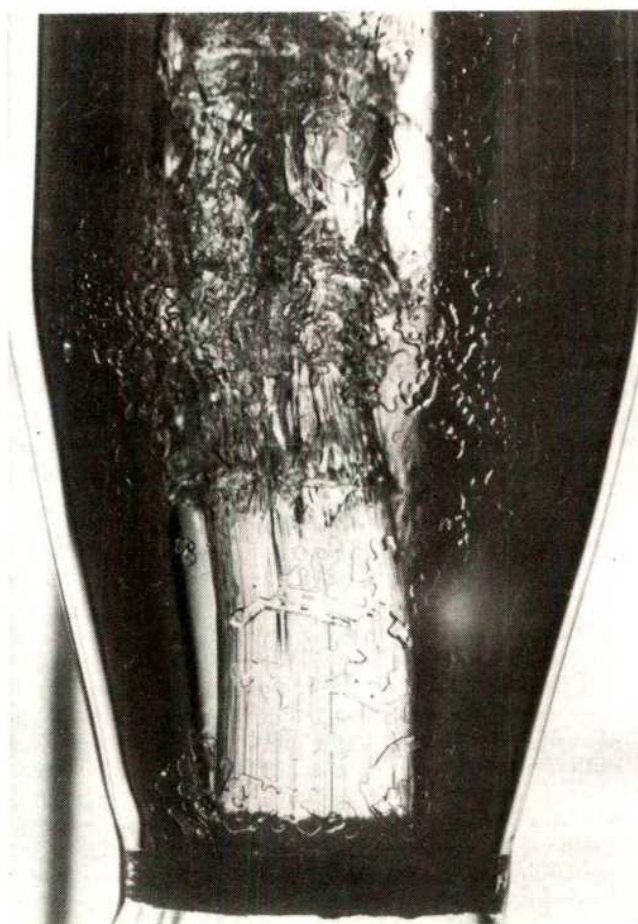


Fig. 2 Photograph taken at condition of no water flow. $T = -0.05^\circ\text{C}$, $p_i - p_w = 63\text{ kPa}$, $p_i = 101\text{ kPa}$.

Decreasing the water pressure at constant temperature, while the pressure on the ice remained at 1 atm, led to a decrease of the rate of water movement and eventually to a reversal of its direction, accompanied by melting of the ice. This is illustrated in Figs 2 and 3; it can be clearly seen that the ice column melts at the bottom.

The pressure difference between ice and water at equilibrium (no water flow) is found to obey closely the equation

$$p_i - p_w = -h(T - T_0)/v_w T_0$$

where the subscripts i and w refer to ice and water, p is pressure, T temperature, T_0 equilibrium temperature at $p_i = p_w = 1\text{ atm}$, h specific enthalpy of fusion, and v specific volume. This equation can easily be found by equating the differentials of the chemical potentials of ice and water and the subsequent



Fig. 3 Photograph taken 1 d after that of Fig. 2. $T = -0.05^\circ\text{C}$, $p_1 - p_w = 76\text{ kPa}$, $p_1 = 101\text{ kPa}$.

integration for constant h and v_w . The temperature range investigated was -0.01°C to -0.06°C .

A full account of this work will be published elsewhere.

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Inaccuracy in rainfall measurement

RAINGAUGES are intended to give accurate measurements of precipitation. There is, however, a growing interest in the chemical composition of rainwater, and in the effects of chemicals in rain on plants and soil. It is also sometimes realised that the water collected in raingauges may be contaminated by dry deposition on the collecting funnels, and also by chemicals derived from the materials used in constructing these funnels and the collecting bottles beneath them.

For this latter reason, raingauges may be constructed of high density polythene, or of polypropylene, materials found not to contaminate seriously the water they collect. The collecting funnels of these raingauges may be cleaned at various intervals by different workers, in an attempt to reduce surface contamination. Unless the cleaning is done in a standard way at a standard time, the chemical analyses of different samples may be difficult to compare. It is fairly

common practice to clean funnels once a week, when samples are being taken. This may mean that in dry weather all surface contamination is lost, while if it rains just before the surface is cleaned much of the soluble material on the funnel's surface will be washed into the collecting bottle.

I now find that cleaning the polypropylene funnels may also affect the volume of the samples of rain collected. Funnels which have been exposed for several months, and which have been washed only by the falling rain, allow all the precipitation falling on them to run quickly down into the collecting bottles. The same happens with dew. These 'dirty' funnels measure the rainfall accurately.

When, however, the funnels are cleaned by daily wiping with paper tissue damped with distilled water, they behave quite differently. Their surface is seen to be covered with beads of water immediately after rain, or in the early morning when there has been a heavy dew fall. This water does not run down into the collecting bottle, and is rapidly evaporated and lost when the weather is dry.

The amount of water lost may not be insignificant. The polypropylene funnels I use have a diameter of 20 cm. This means that they collect 31.4 ml of water for each mm of rainfall. Shortly after an occasion where there was a rainfall of 0.8 mm, the 'dirty' funnels were almost completely dry, having transferred 25 ml of water to their collecting bottles. The 'clean' funnels were covered with drops of water. These were mopped up with paper tissues, which were then weighed. The water on the funnels was found to amount to between 5 and 6 ml. With this slight amount of rain the shortfall in collection amounted to some 20%. In heavy rain the loss is not, proportionately, so great, but it is still significant.

This behaviour of the raingauge surface will not only affect the volume of water collected; it will also affect the way in which the surface deposition is dissolved. A heavy dew or a slight shower which runs off the 'dirty' funnel will remove much soluble deposition, while on a 'clean' funnel, if the dew or rain is not collected, any salts dissolved will be redeposited when the water evaporates, and they will not appear in the sample which is collected and analysed.

It thus seems that, if we are interested only in accurate measurements of the amount of rainfall, we should not clean the surfaces of our collecting funnels. If we are interested in rainwater chemistry, we should plan our work knowing that the results may be affected by the treatment given to the collecting surfaces.

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Gas velocities inside a burning cigarette

To understand more fully the factors that contribute to the formation and mass transfer of products within a burning cigarette, and to specify the type of reactions occurring, it is necessary to know the velocity of gases inside the combustion coal. In this study, velocities have been calculated from pressure and temperature distributions inside the burning cigarette. Gas velocities as high as 400 cm s^{-1} occur within the coal during a puff. Thus the residence times of gases in the coal can be much lower than 1 ms. This time is not long enough to allow molecular reactions of primary products in the gas phase to contribute to the final products.

The direction of the flow of gases inside the cigarette can be obtained from the internal pressure distribution, since the gas flow is perpendicular to the pressure contours and is towards

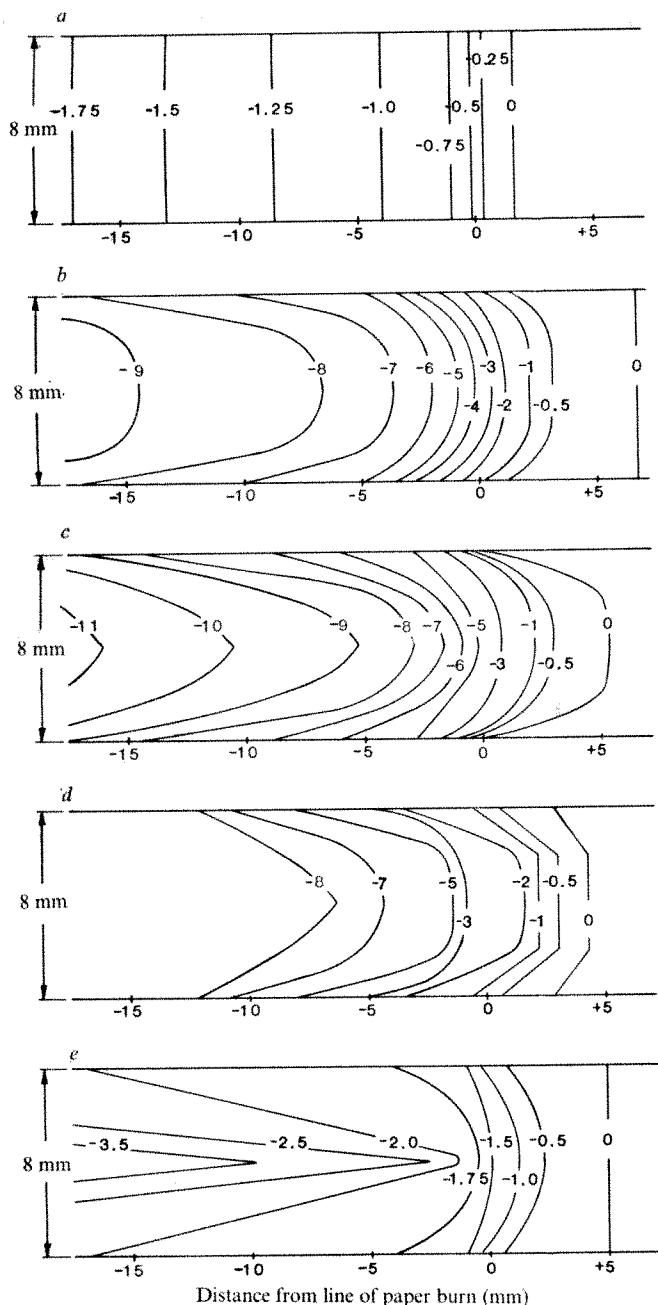


Fig. 1 Pressure (cm water) distribution in the cigarette at various times after the start of a 2-s, 35 cm³ puff (with a square pressure-time puff profile). Axial positions in the unburnt tobacco rod are quoted as negative distances from the burn line, while positions in the coal and ash are quoted as positive distances. *a*, 0.1 s; *b*, 0.5 s; *c*, 1.0 s; *d*, 2.0 s; *e*, 2.1 s.

lower pressures. Furthermore, inside a cigarette the volumetric flow of gases (V , cm³ s⁻¹) and pressure differential (ΔP , cm water) across a given length (L , cm) obey Darcy's Law¹

$$V = -\frac{1}{\epsilon} A \frac{\Delta P}{L} \quad (1)$$

where A is the area through which the gas flows (cm²), ϵ is the impedance of the tobacco bed at room temperature (cm water s cm⁻²), and 1 cm water = 98 N m⁻².

The impedance of a body is directly proportional to the viscosity (η , poise) of the fluid flowing through the body². A previous study has confirmed that the structure of the tobacco bed does not change significantly with temperature¹, so that ϵ is a function of η only. Consequently, if the flowing gas is at

temperature T , from equation (1) the component of velocity u_x (cm s⁻¹) in any direction is given by

$$u_x = -\frac{1}{\epsilon} \frac{\eta_0}{\eta_T} \frac{\partial P}{\partial x} \quad (2)$$

where η_0 and η_T are the viscosities of the gas at room temperature and T , respectively.

Thus, since the temperature distribution of the gas phase inside a burning cigarette is known³, the gas velocities inside the cigarette can be obtained from the pressure distribution using equation (2), assuming that the viscosity of the gases inside the cigarette vary with temperature as does air. This assumption is not critical, since the major gas phase components inside the cigarette (nitrogen, water, carbon monoxide, carbon dioxide and oxygen) all have viscosities whose variation with temperature closely parallels that of air^{4,5}.

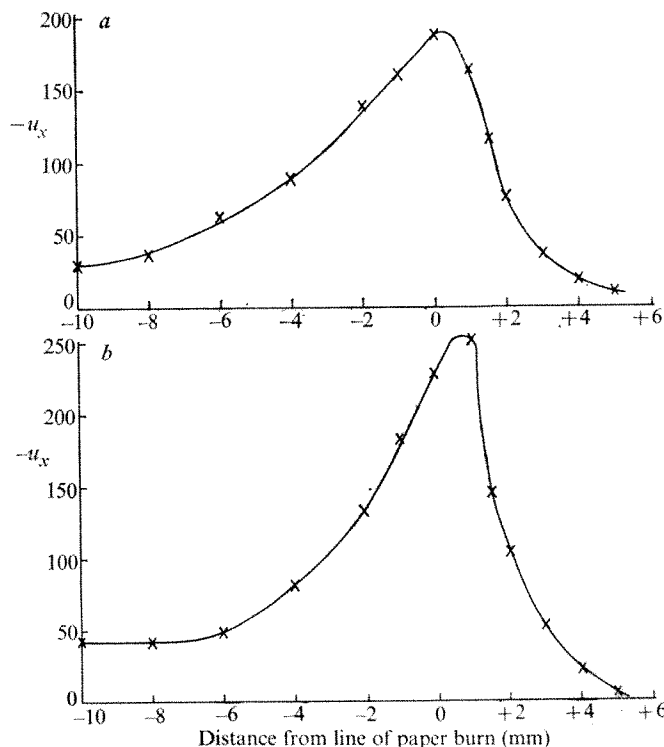
The pressure distribution inside the cigarette during the third puff of the smoking cycle has been determined. A quartz glass tube, 0.80 mm internal diameter, 1.12 mm outer diameter, and 35 mm long, was inserted radially into the cigarette, while its other end was connected to an Ether type UP1 pressure transducer. The output from the pressure transducer was monitored on a CAMAC data logger. The resolution of a pressure measurement was ± 0.01 cm water. The transducer-probe system gave 100% response to a pressure change from ± 10 cm water (relative to atmospheric pressure) to atmospheric pressure in under 0.1 s.

The cigarettes used, and the experimental and computational techniques, were similar to those described previously for determining the temperature distributions³.

The pressure contour distributions during the puff are shown in Fig. 1. The mean pressures have 95% confidence limits of up to 25% of their value, due to slight variations in the cigarettes used. Consequently, the quoted pressure distributions are very much mean distributions, and the distribution in an individual cigarette could be significantly different.

The mean pressure contours 0.1 s after the start of the puff are all perpendicular to the cigarette axis (Fig. 1*a*). Thus, the

Fig. 2 Gas velocity (u_x , cm s⁻¹) along the central axis of the cigarette at *a*, 0.5 s; and *b*, 1.0 s after the start of a 2-s puff. The cigarettes had an impedance of 0.042 cm water s cm⁻².



gas flow through the cigarette is entirely parallel to the cigarette axis. Furthermore, the flow only extends to a distance of 1.5 mm in front of the line of paper burn.

At 0.5 s after the start of the puff, the pressure within the cigarette decreases further, air leakage through the paper causes convex shaped contours behind the coal, measurable negative pressures extend for up to 6.6 mm in front of the paper burn line, and the pressure contours in the coal are convex (Fig. 1b). Consequently, at this time in the puff, a proportion of the air enters the coal in an approximately radial direction, just in front of the paper burn line. As the puff progresses (Fig. 1), the pressure contours in the vicinity of the paper burn line tend to become more nearly parallel to the

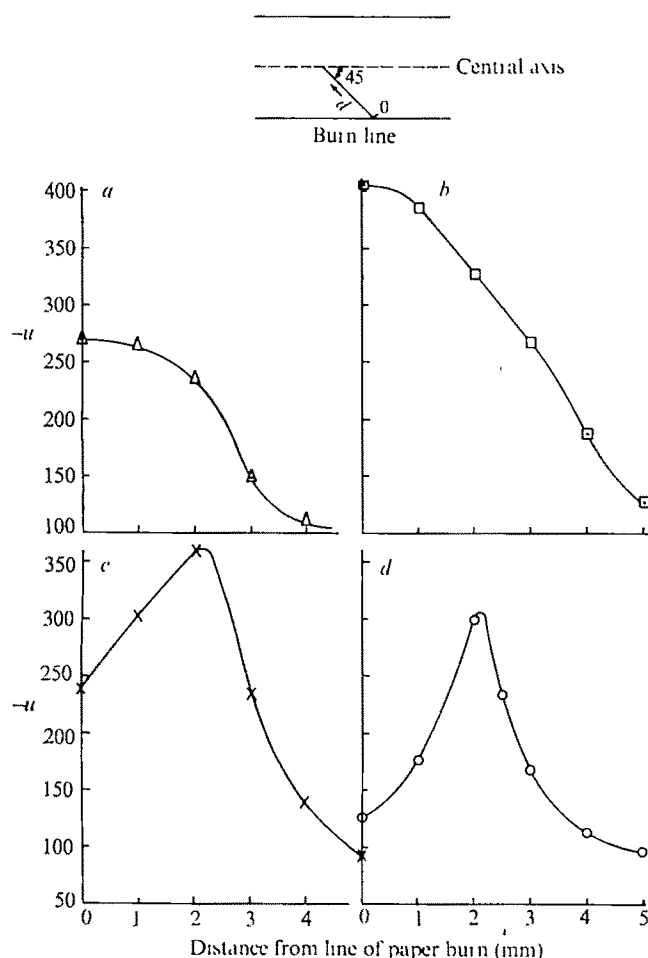


Fig. 3 Gas velocity (u , cm s⁻¹), d mm from the paper burn line, along a line at 45° to the central axis, at various times during a 2-s puff: a, 0.5 s; b, 1.0 s; c, 1.5 s; d, 2.0 s.

cigarette axis, indicating that the air influx in this region becomes more nearly radial. Thus, during a puff, air initially enters the coal in a radial direction in the vicinity of the paper burn line, and its line of flow gradually becomes axial behind the coal. Consequently, it is largely the peripheral region of the coal that burns during a puff, as has previously been observed by direct studies⁶.

The pressure distribution 0.1 s after the end of the puff is shown in Fig. 1e. The pressures inside the cigarette have increased considerably (become less negative) in the ensuing 0.1 s, particularly at the peripheral regions of the cigarette behind the paper burn line. Thus, when the puff ends, air must enter the cigarette predominantly through the paper. Within 1 s of the puff ending, no mean negative pressures are observed within the cigarette.

The gas velocities along the central axis of the cigarette during a puff, calculated using equation (2) and data from Fig. 1 and ref. 3, are shown in Fig. 2. At distances greater than 20 mm behind the line of paper burn, where the flowing gases are close to room temperature, the linear gas velocity along the central axis is 30–42 cm s⁻¹ at all times during the puff. The greatest linear velocities along the central axis (160–250 cm s⁻¹) are obtained just in front of the line of paper burn (Fig. 2).

Gas velocities in any direction of interest can be calculated. Inspection of Fig. 1 suggests that a major route for gas intake into the coal throughout the puff is at an angle of 45° to the central axis, and intersecting the paper at the burn line. Calculated velocities along this line are given in Fig. 3, and it is seen that 1.0 s after the start of the puff, gas velocities in excess of 400 cm s⁻¹ are obtained. Along this line, 1.0 s after the start of a puff, the gas temperature falls from 520 °C at the burn line to 100 °C 1.4 mm away³. Consequently, the incoming gases have been heated to 520 °C, and then cooled to 100 °C, in 350 μ s. Molecular reactions of intermediate volatile products are almost certainly too slow to occur in the residence times available in the hot coal. This means that further reaction of primary decomposition and combustion products in the gas phase must occur by radical reactions and possibly by ionic reactions as well.

It is interesting to note in Figs 1 and 2 that measurable pressures and central axis gas velocities only extend to distances of about 5 mm in front of the paper burn line during the puff. Hot gases (temperatures greater than 100 °C), however, extend to distances of 11–13 mm in front of the paper burn line³. Consequently, diffusion processes, and not forced flow processes, are the major mechanism by which the mass transport of gases occurs in the coal at distances greater than about 6 mm from the paper burn line.

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Dependence of induced mutation on spontaneous mutation in *Tribolium castaneum*

In plants the effect of physical and chemical mutagens has been found to depend on the frequency of spontaneous mutation^{1,2}, but no such information is available from animal material. I have therefore investigated the relationship between spontaneous and X-ray-induced somatic mutation in the flour beetle *Tribolium castaneum*. This relationship was found to be positive and significant when the genotype was manipulated by artificial selection for speed of development.

The criterion used consisted of the wing developmental abnormalities that are under genetic control (21 genes have been identified, all with a recessive mode of inheritance³) and are subject to environmental modification^{3–5}. The data are the same as those for the lines selected for speed of

development⁴. Beetles were irradiated as 1-d-old pupae with 10 kR and the induced somatic mutations were observed in the adults which emerged. Spontaneous mutations were observed in non-irradiated samples. The experimental conditions were maintained at 33 °C and 70% relative humidity.

Figure 1 shows that the rate of induced mutation was dependent on that of spontaneous mutation among wild, slow and fast lines of beetles. The degree of determination of R^2 is very high (0.994), indicating that variation in the induced mutations could be explained by variation in their spontaneous rates.

Because the rate of mutation for wing abnormalities is under genetic control and is subject to natural and artificial selection^{5,6}, both types of mutation could be an expression of common underlying processes in the enzymatic system acting on the regulatory mechanism which controls DNA synthesis and repair⁷. The slow line is expected to have the lowest rate of both spontaneous and induced mutation, for slowing of development gives more time for repair to take place⁴. The wild type, the best fit genotype, has higher mutation rates than both the selected strains. These results confirm that the optimal mutation rate within wild strains is favoured by natural selection⁸ and is independent of the rate of lethal mutation, as a high mutation rate is expected to have deleterious effects⁹. On the other hand, the relationship between fitness (% adult emergence) and mutation rate (% wing abnormalities) for eight wild strains of *T. castaneum* was negative¹⁰ ($r = -0.82$, $P < 0.05$).

A line selected for intermediate development values (stabilising selection) showed a higher degree of spontaneous mutation and a slower degree of induced mutation than did

Fig. 1 Relationship between X-ray-induced mutations and spontaneous mutations of three lines selected for seven generations for speed of development and their control line. Means \pm s.e. are based on four replications. Correlation and regression coefficients are based on the angular values of the means of the fast, slow and control lines (connected by the straight line). Analysis of variance on such values indicated highly significant differences between lines and between the two types of mutations, but not between replications nor sex or for the interaction components. Numbers are the total number observed. $r = 0.997$; $P = 0.05$; regression coefficient = 3.275, s.e. = 0.242.

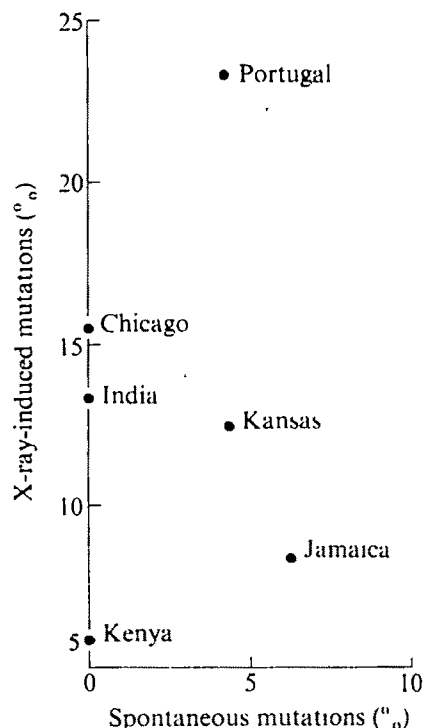
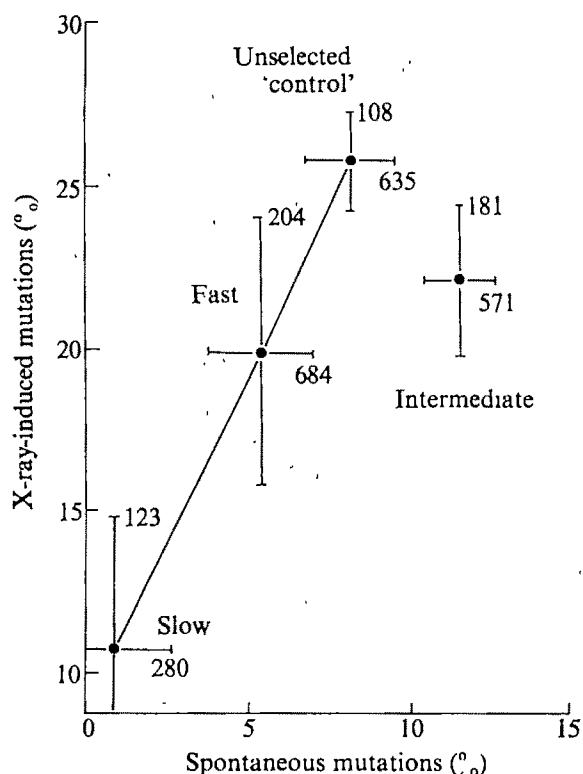


Fig. 2 Scatter diagram for the relationship between the two types of mutations of six wild populations of *T. castaneum*.

the control line (Fig. 1). This indicates that stabilising selection is efficient in producing genotypes that could stand sudden environmental changes (X ray), while these genotypes are less stable in normal environmental conditions. In other words, X rays have more effect on the wild strain (due to a certain degree of homozygosity) than the intermediate line (due to developmental homeostasis), while in stable environments spontaneous mutation is more pronounced in the intermediate line (due to induction of new recessive mutants) than the wild strain (due to greater genetic homeostasis). The different degrees of genetic and developmental homeostasis among different wild populations with different genetic backgrounds will undoubtedly affect the relationship between spontaneous and induced mutations observed among these populations (Fig. 2).

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Artificial deciliation causes loss of calcium-dependent responses in *Paramecium*

WHEN a paramecium collides with an obstacle, it reverses the direction of the effective stroke of its cilia, swims backwards for a while, and, with one or more repetitions of this 'ciliary reversal' response, avoids the obstacle¹. When the medium is disturbed² or when the posterior part of its body is stimulated mechanically, it swims faster than usual and

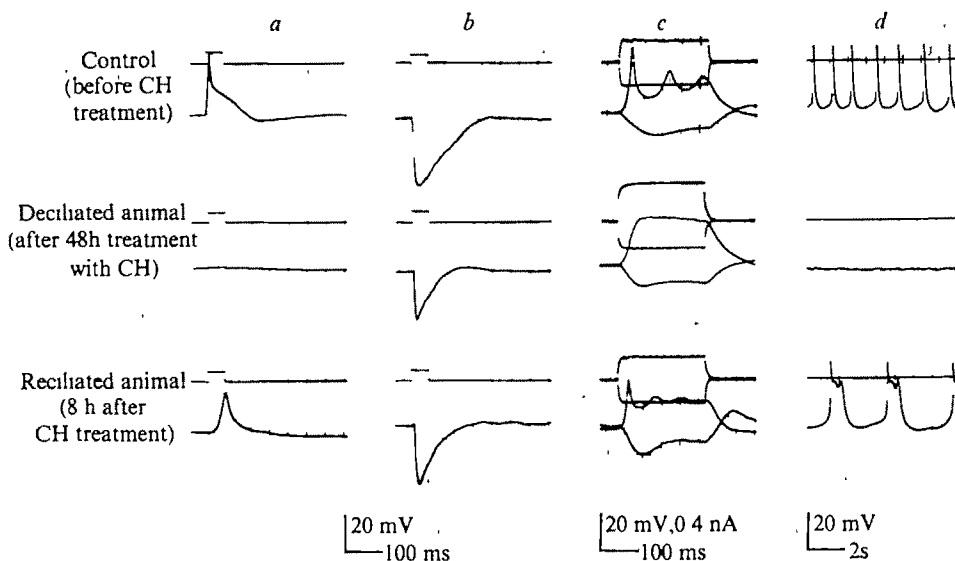


Fig. 1 *P. caudatum*, reared in hay infusion and washed in the adapting solution, which consisted of 2 mM CaCl_2 , 1 mM KCl and 1 mM Tris-HCl, pH 7.4, was deciliated with CH, which was added to the adapting solution at 5 mM. The animals were kept for about 48 h in this solution at 20°C . *a*, *b*, and *c*, Membrane potentials were recorded in the adapting solution at $20 \pm 2^\circ\text{C}$; *d*, recordings were made in a solution of 2 mM BaCl_2 , 1 mM CaCl_2 and 1 mM Tris-HCl, pH 7.4, $20 \pm 2^\circ\text{C}$. For recording, a glass capillary microelectrode, filled with 0.3 M KCl and with a resistance of 200–400 M Ω was used. Specimens that showed gradual depolarisation of the resting membrane potential were discarded. Successful recordings were made from about 120 specimens. *a*, Responses to mechanical stimuli given to the anterior end of the animal by means of a glass stylus with a rounded tip mounted on a piezoelectric phonograph cartridge. In each record the upper trace indicates the zero potential level as well as the electric pulse used to drive the stylus. *b*, Responses to mechanical stimuli given to the posterior end. The methods of stimulation and recording are the same as in *a*. *c*, Responses to direct current pulses applied through an intracellular electrode. The upper traces in each record indicate both the zero potential level and the applied current. In the case of the deciliated animal, the current was increased to exclude the possibility of change in the threshold of the regenerative response. *d*, Spontaneous membrane activity in Ba^{2+} - Ca^{2+} mixture. The upper trace in each record shows the zero potential level.

escapes from the stimulus mainly by increasing the frequency of ciliary beat. The current view of the underlying mechanisms can be summarised as follows^{3,4}. A mechanical stimulus to the anterior end of the animal deforms the cell membrane of this region, increasing the permeability to Ca^{2+} and causing a depolarisation which further activates the Ca^{2+} -channels in the membrane. Thus, the depolarisation develops regeneratively towards the equilibrium potential of Ca^{2+} . The Ca^{2+} which has entered the cell acts on some mechanism controlling the direction of ciliary beat to bring about ciliary reversal. On the other hand, a stimulus to the posterior end activates the K^{+} -channels in the membrane, causing hyperpolarisation and an increase in ciliary beat frequency. Although the animal behaves as a mechanoreceptor cell in the early stages of these events, it is not clear which part of the cell transduces the mechanical stimulus to the activation of Ca^{2+} - or K^{+} -channels. We report here the effect of removal of cilia on the electrophysiological responses of paramecium to mechanical as well as some other stimuli.

Paramecium caudatum was deciliated by treatment with chloral hydrate (CH) according to the method of Kuźnicki⁵. The methods of stimulation and recording were similar to those of Naitoh and Eckert⁶. Typical results are shown in Fig. 1.

Figure 1*a* and *b* shows membrane responses to mechanical stimuli, given by a fine glass stylus to the anterior and posterior ends of the animal, respectively. Untreated paramecia responded with depolarisation to anterior stimulation and with hyperpolarisation to posterior stimulation. When the animals were incubated in medium containing 5 mM CH, they lost the ability to swim within 24 h and sank to the bottom of the incubating glassware. Under a light microscope, the cilia appeared unimpaired in both number and individual length at this stage, but their movement was uncoordinated. Electrophysiological responses to mechanical stimulation were not significantly different from those of the untreated animal. As incubation continued, the cilia began to drop off, and in about 48 h all were lost except for

those in the oral groove. The deciliated paramecia retained the hyperpolarising response to posterior stimulation, but did not show a depolarising response to anterior stimulation. A stronger stimulus (obtained by pushing the stylus from a shorter distance) caused hyperpolarisation even if given to the anterior end. This eliminates the possibility of a general decrease in responsiveness. The resting membrane potential was not affected by CH. If a deciliated paramecium was transferred to medium containing no CH, cilia regenerated completely in 6–8 h. The reciliated paramecia responded with depolarisation to anterior stimulation, although both the amplitude and the rate of increase of the response were smaller than normal for some hours after the apparent completion of reciliation.

The finding that the depolarising responses disappear and reappear in parallel with the loss and the regeneration of cilia can be interpreted most readily if it is assumed that the Ca^{2+} -channels are localised in the ciliary membrane, whereas the K^{+} -channels are not. Although the possibility cannot be ruled out that CH inhibits Ca^{2+} -channels in the extraciliary membrane independently of the deciliating effect, it seems unlikely that the time course of such inhibitory effects coincides with that of the deciliating effect. The interpretation that the Ca^{2+} -channels are lost by deciliation is supported by two further experiments.

Figure 1*c* shows responses to current pulses applied through a second intracellular microelectrode. Fluctuations in potential (peaks) superimposed on the passive depolarisation are seen in the untreated animal. These have been ascribed to the regenerative activation of Ca^{2+} -channels⁷. This response also disappeared with deciliation and reappeared with reciliation. Similar results have recently been reported by Dunlap and Eskert^{8,9} who found that deciliation by vigorous agitation of *P. caudatum* incubated in CH eliminates the 'calcium response' to a depolarising current pulse, while regrowth of the cilia restores the response. They also interpret the results as indicating the localisation of Ca^{2+} -channels in the ciliary membrane.

Figure 1*d* shows spontaneous periodic depolarisations

observed in a solution containing Ba^{2+} and Ca^{2+} . This response is believed to depend on Ba^{2+} moving into the cell through the Ca^{2+} -channels¹⁸. The Ba^{2+} -response also disappeared and reappeared in parallel with deciliation and reciliation, although the frequency and the form of potential changes were altered somewhat in the reciliated animal.

Electron microscopic studies on paramecia have shown that binding sites for divalent cations are localised in the basal region of each cilium¹¹⁻¹³. Plattner suggested that the granular patches of these divalent cation-binding sites have a role in the regulation of ciliary movement by binding and releasing Ca^{2+} . It is interesting that with CH treatment the cilium is lost just proximal to these structures¹⁴. Although there is no evidence that these Ca^{2+} -binding sites are identical with the Ca^{2+} -channels, it is tempting to relate our results to these morphological findings.

During the preparation of our manuscript we learnt that Dr K. Dunlap, University of California, Los Angeles (personal communication), also observed, in *Paramecium caudatum*, loss of the regenerative component of membrane response by CH deciliation and its recovery with reciliation.

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Two populations of heterozygote erythrocytes in moderate hypoxanthine guanine phosphoribosyltransferase deficiency

THE X-linked condition hypoxanthine guanine phosphoribosyltransferase (HGPRT) deficiency may be either gross or moderate in degree and is invariably associated with overproduction of urate in the hemizygote¹⁻³. In addition, however, the gross deficiency state (Lesch-Nyhan syndrome)³ also manifests with mental deficiency, choreoathetosis, spasticity and self-mutilation. Fibroblasts from heterozygotes for the gross deficiency state display mosaicism, some having normal HGPRT activity and others the reduced HGPRT activity characteristic of the hemizygote⁴. However, HGPRT activity in erythrocyte lysates from such heterozygotes is normal. Heterozygotes for the moderate HGPRT deficiency state, on the other hand, have HGPRT activity in erythrocyte lysates ranging between 20% of normal and completely normal values⁵. To elucidate this, we have developed an autoradiographic procedure to show HGPRT activity in individual erythrocytes. In families with moderate HGPRT deficiency, in whom erythrocytes of the hemizygote were not significantly labelled by this procedure, heterozygotes showed two erythrocyte populations, one deficient in HGPRT and one containing HGPRT activity. On the other hand, in families whose HGPRT deficiency was gross and manifested as the Lesch-Nyhan syndrome, heterozygotes showed only the normal pattern of labelling. Thus, mosaicism was present in erythrocytes from heterozygotes for moderate, but not for gross, HGPRT deficiency. The former

finding supports the Lyon hypothesis⁶ whereas, the latter finding suggests that either the X chromosome carrying the mutant HGPRT gene is preferentially inactivated, or the X chromosome is randomly inactivated with later selection against the erythrocyte precursors containing the mutant HGPRT enzyme.

Normal nucleated cells incorporate tritiated purine bases into nucleic acids, permitting autoradiographic demonstration of the presence of HGPRT activity⁷. Erythrocytes also contain considerable amounts of HGPRT and, although they are capable of accumulating significant quantities of labelled mononucleotides, they do not synthesise nucleic acids. Earlier attempts to demonstrate HGPRT activity in erythrocytes by autoradiography failed because the soluble mononucleotides, unlike nucleic acids, are lost immediately the selective permeability of the cell membrane is destroyed by fixing or drying the cells. Thus, the demonstration of HGPRT activity in erythrocytes by autoradiography using the conversion of tritiated hypoxanthine to nucleotide required fixation of the labelled inosinic acid *in situ*. We have done this by precipitation of the mononucleotides by lanthanum, as developed by Bakay⁸, simultaneously with fixation of the red cells by osmium tetroxide. After autoradiography, silver grains were counted over 200 cells and the distribution of the grain counts per cell was plotted. The distribution of counts in normal subjects showed that at least 90% of erythrocytes had more than five grains per cell; in Lesch-Nyhan subjects, no erythrocytes had more than this value. The grain counts per cell were therefore combined into three groups (Fig. 1). To validate the method, the distribution of grain counts was studied in artificial mixtures of erythrocytes from a normal subject and one with the Lesch-Nyhan syndrome. The results enabled the proportions of the two types of cell to be confirmed (Fig. 1) and thereby justified the search for two populations of erythrocytes in heterozygotes.

Members of one family with the Lesch-Nyhan syndrome and three families with less severe HGPRT deficiency were studied by this technique (Fig. 1). The HGPRT activity in cell-free lysates of erythrocytes and fibroblasts from hemizygotes and heterozygotes for each of these families is shown in Table 1. In family W (Lesch-Nyhan syndrome)⁹, erythrocytes from two obligate heterozygotes, both of whom showed normal activity in an erythrocyte lysate, showed a uniform pattern of labelling indistinguishable from normal. In hemizygotes from families L and C¹⁰, erythrocytes were indistinguishable from those of the Lesch-Nyhan syndrome. The heterozygote in family L, whose HGPRT activity in an erythrocyte lysate (Table 1) indicated that, if there were two populations, 90% of her erythrocytes would contain mutant HGPRT enzyme^{*}, showed 86% of

Table 1 Hypoxanthine phosphoribosyltransferase activity in cell lysates (nmol per mg protein per h)

Family		Erythrocytes	Fibroblasts
W (Lesch-Nyhan)	Hemizygote	< 0.01	< 1
	Heterozygote 1	89	70
	Heterozygote 2	85	88
L	Hemizygote	< 0.05	7
	Heterozygote	10	103
C	Hemizygote	< 0.05	27
	Heterozygote	44	46
B	Hemizygote	13	30
	Heterozygote	18	60
Normal \pm s.d.		101 \pm 12	157 \pm 32

*Percentage of erythrocytes containing mutant HGPRT enzyme =

$$\frac{A_N - A_H}{A_N - A_M} \times 100$$

where A_N is HGPRT activity in normal erythrocyte lysate, A_M is activity of mutant HGPRT enzyme in erythrocyte lysate from hemizygote, and A_H is HGPRT activity in erythrocyte lysate from heterozygote

erythrocytes with less than five grains per cell. In the heterozygote from family C, the expected percentage of HGPRT-deficient erythrocytes was 56% and a value of 70% was observed. These results were taken to confirm the presence of two populations of erythrocytes in heterozygotes for the moderate HGPRT deficiency syndrome.

The fourth family, B, had the least severe HGPRT deficiency (Table 1) and the least severe clinical manifestations¹⁰. However, the pattern of grain counts over the erythrocytes of the hemizygote was close to normal, although there were more cells with less than five grains per cell than were seen normally. The autoradiographic procedure was therefore modified by reducing the duration of incubation and extending the exposure time. This modification did not significantly alter the pattern of labelling in normal erythrocytes, but it resulted in 86% of the erythrocytes from the hemizygote showing less than five grains per cell. Erythrocytes from the heterozygote of this family also showed a similar proportion of HGPRT-deficient cells, a finding expected from the HGPRT activity of her erythrocyte lysate. This technique has therefore demonstrated the expected

proportion of normal and HGPRT-deficient erythrocytes in heterozygotes from families with moderate HGPRT deficiency. It would seem that in the functionally less severe HGPRT mutations, random inactivation of the X-chromosome occurs and two types of erythropoietic clones result, one of which produces normal erythrocytes and another which produces erythrocytes containing mutant HGPRT enzyme.

In the severe HGPRT deficiency of the Lesch-Nyhan syndrome, on the other hand, although the technique could identify the reduced HGPRT activity of the mutant enzyme, no HGPRT-deficient erythrocytes were detected in two heterozygotes whose heterozygosity had been established by both the production of affected sons and autoradiography of fibroblasts. Although two populations of fibroblasts are found in heterozygotes for the Lesch-Nyhan syndrome, all previous studies have demonstrated normal HGPRT activity in erythrocyte lysates, and several other studies have suggested that the X chromosome of all of their erythrocyte precursors was normal¹¹⁻¹³. The present technique provides direct confirmation of this. Either inactivation of the X chromosome is not random in these cells

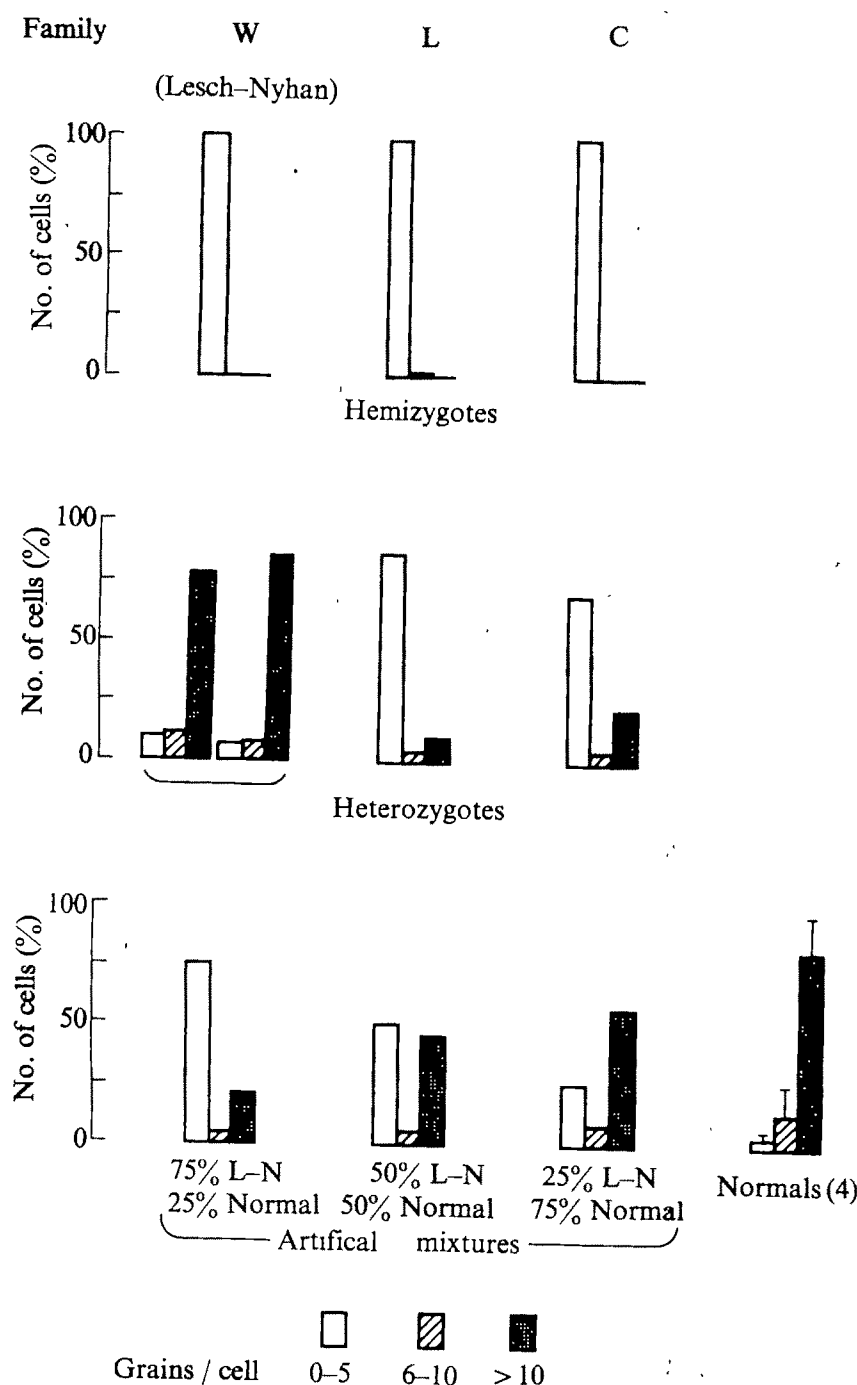


Fig. 1 Comparison of grain counts per cell in autoradiographs of erythrocytes from normal subjects, from hemizygotes and heterozygotes from three families with HGPRT deficiency and in artificial mixtures of normal and HGPRT-deficient erythrocytes. Erythrocytes were incubated with labelled precursor in a special incubation chamber constructed by cementing a glass ring to a cover slip with Araldite. The incubation mixture contained 0.05 M sodium phosphate (pH 7.4), 0.075 M sodium chloride, 5 mM glucose and 0.05 mM ³H-hypoxanthine [1 Ci mmol⁻¹ (Amersham) 99% pure on electrophoresis]. Aliquots (200 μ l) of the incubation mixture were pipetted into the incubation chamber, and 5 μ l of a 1:100 saline dilution of heparinised and washed packed erythrocytes was added and mixed by swirling. An acid-washed glass microscope slide was then placed over the incubation chamber, and the whole slide and chamber were then inverted and incubated in an humidified atmosphere at 37°C for 30 min. During the incubation, the cells settled and became firmly attached to the slide; the glass ring and coverslip prevented spreading of the cell suspension. The wide separation of cells on the slide also prevented any possible cell-to-cell transfer of labelled material. After incubation, the chamber was removed and the slide immediately washed thoroughly to remove all traces of labelled hypoxanthine, care being taken to cover the cells at all times with a layer of saline. After washing, the slides were flooded with a fixative consisting of 1 volume of 0.1% osmium tetroxide and 9 volumes of 0.12 M neutral lanthanum chloride (0.12 M lanthanum chloride neutralised with lanthanum oxide and filtered). After fixation for 15 min, the slides were washed repeatedly with distilled water and allowed to dry. The dry slides were immersed in absolute methanol for 1 h, washed with methanol and dried. The slides were then coated with photographic emulsion (Kodak NTB 2) and exposed for 3 d. After development, the slides were stained with 0.5% aqueous eosin for 10 min, washed with water, destained in a solution containing 50% methanol and 50% citrate phosphate buffer (4 volumes 0.1 M citric acid + 6 volumes 0.2 M disodium hydrogen phosphate) for about 15 s and immediately washed with absolute methanol and dried. The autoradiographs were permanently mounted before grain counting. Control slides using normal erythrocytes were examined with each batch of cells studied.

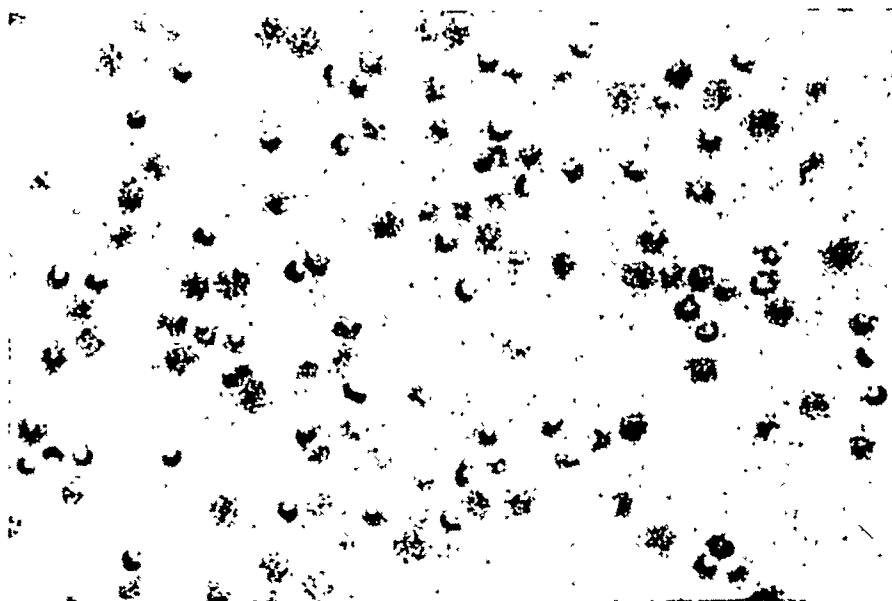


Fig. 2 Autoradiograph of erythrocytes from the heterozygote of family C showing populations of labelled and unlabelled cells.

or there is subsequent selection against haemopoietic precursor cells containing mutant HGPRT enzyme. Support for such selection against HGPRT-deficient stem cells *in vivo* in heterozygotes for the Lesch-Nyhan syndrome, has been provided by studies of the culture characteristics of their bone marrow cells¹⁴. Our findings further suggest that, whereas there exists a wide spectrum of severity of HGPRT deficiency, there is a critical intracellular level of HGPRT activity which, in the hemizygote, leads to the Lesch-Nyhan syndrome and which, in the heterozygote, renders affected erythrocyte precursors at a marked functional disadvantage in comparison with the normal.

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Glucocorticoid receptors in murine embryonic facial mesenchyme cells

In various experimental animals, clefting of the developing secondary palate can be induced by the administration of glucocorticoids to pregnant animals¹⁻⁴. Various inbred strains of mice exhibit different degrees of susceptibility to glucocorticoid-induced cleft palate⁵⁻⁷. Specifically, A/J mice

treated with glucocorticoids between days 11 and 14 of gestation (day of detection of vaginal plug designated day 0 of pregnancy) produce 100% of their offspring with cleft palate, whereas C57BL/6J (C57) mice similarly treated produce offspring with only 20-25% cleft palate. Although it has been demonstrated that glucocorticoids can elicit divergent physiological responses in various differentiated cell types through a class of specific cytoplasmic and nuclear receptor proteins⁸⁻¹¹, the exact molecular mechanism(s) by which glucocorticoids function as teratogens as well as the aetiology of the strain-specific difference in the response to glucocorticoids are unknown. We present here evidence that mouse facial mesenchyme cells obtained either directly or in primary culture possess specific glucocorticoid-receptor proteins of high affinity and limited capacity for dexamethasone. Furthermore, we report that A/J facial mesenchyme cells possess approximately double the amount of receptor proteins than C57 mesenchyme cells.

Embryos were obtained from day 14 pregnant A/J or C57 mice (purchased timed pregnant from Jackson Laboratories and obtained on day 11 of pregnancy). Embryos were dissected from the uteri in phosphate-buffered saline (PBS). Placentae and other extraembryonic membranes were removed, and the embryos were washed in PBS. Secondary palates or maxillary processes were dissected from the embryos and pooled. Minced tissues were dissociated with 0.25% trypsin (Difco) containing 0.1% EDTA (Fisher Scientific) for 10 min at 37 °C on a shaking water bath. After dissociation, NCTC-109 medium (Microbiological Associates) containing 10% foetal calf serum (Gibco), streptomycin (100 µg ml⁻¹, Gibco) and penicillin (100 units ml⁻¹, Gibco) was added at 4 °C to inhibit the trypsin. Cells were dispersed, washed and seeded into 75-cm² tissue culture flasks (Falcon) at a density of 5.4 × 10⁴ cells per cm². Cells were grown in NCTC-109 medium containing 10% foetal calf serum and antibiotics. Cultures of mesenchyme cells obtained from either the secondary palate or maxillary processes appeared to have a fibroblastic morphology.

Cell suspensions were incubated for 40 min at 37 °C with ³H-dexamethasone to measure glucocorticoid receptor activity since fluorinated glucocorticoids in contrast to the naturally occurring corticosteroids do not bind to serum transcortin (CBG)¹², a component of foetal calf serum. Under these conditions, steroid uptake was maximum for both A/J and C57 mesenchyme cells. Figure 1 illustrates one of several experiments in which the specific binding of ³H-dexamethasone to whole cell suspensions of A/J or C57

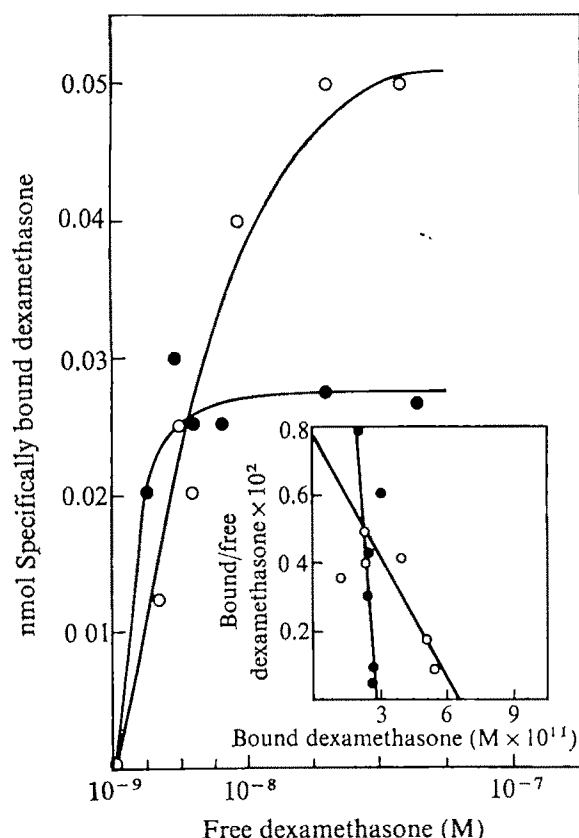


Fig. 1 At confluency (approximately 4–5 d after seeding), cells (1.5×10^6 per cm^2 at confluency) were trypsinised (0.1% trypsin), centrifuged and washed in Dulbecco's modified PBS at 4°C (Ca^{2+} and Mg^{2+} free, 0.01% EDTA, 0.02% dextran). Glucocorticoid-receptor activity of both cytoplasmic and nuclear origin was measured in whole cell suspensions using 1, (2)- ^3H -(N)-dexamethasone (specific activity 28 Ci mmol^{-1} ; Amersham/Searle) as a probe according to the method of Baxter *et al.*¹² as modified by Sibley and Tomkins¹³. Approximately 3×10^6 cells obtained from A/J (○) or C57BL/6J (●) maxillae were incubated in 0.5 ml warm (37°C) serum-free NCTC-109 medium containing $1\text{--}60 \text{ nM}$ ^3H -dexamethasone in the absence or presence of an excess of unlabelled dexamethasone (10^{-6} M) in parallel assays to correct for nonspecific binding¹⁴. Cell suspensions were incubated at 37°C for 40 min on a shaking water bath and vortexed periodically. At the end of the incubation, aliquots were removed from each assay to calculate the total concentration of ^3H -dexamethasone, and the suspensions were chilled to 4°C . All subsequent procedures were carried out at 4°C . The cells were centrifuged at $800g$ for 5 min. The pellets were washed with 5.0 ml of PBS, recentrifuged, and resuspended in 0.5 ml of distilled water. The pellets were then freeze-thawed three times in an ethanol-dry ice bath. Aliquots of the lysates were either taken in duplicate and dissolved in 10 ml of scintillation fluid (Hydromix, Yorktown Research) and counted in a Packard Tri-Carb 3375 liquid scintillation spectrometer with an efficiency of 33% for ^3H to calculate the total amount of specifically bound ^3H -dexamethasone, or analysed for total cellular protein by the method of Lowry¹⁵ using bovine serum albumin as a standard. Inset, Scatchard plots¹⁶ of the binding data for ^3H -dexamethasone by suspensions of A/J (○) or C57BL/6J (●) maxillary mesenchyme cells.

primary mesenchyme cells (maxillary) was measured as a function of the free steroid concentration. The curves indicate the presence of specific corticosteroid receptors, for dexamethasone in both cell types, which approach saturation between $5 \times 10^{-8} \text{ M}$ and $2.5 \times 10^{-7} \text{ M}$ dexamethasone. Scatchard plots of the data (inset, Fig. 1) are linear, indicating the presence of a single class of binding sites for dexamethasone in both A/J and C57 mesenchyme cells with an apparent K_D for dexamethasone of $\sim 8.5 \times 10^{-8} \text{ M}$ and $1.3 \times 10^{-7} \text{ M}$ for A/J and C57 cells, respectively; and with 121 fmol of ^3H -dexamethasone bound per mg total cell protein and 54 fmol per mg cell protein for the two cell types. Table 1 represents cumulative data obtained from Scatchard plots¹⁶ from several experiments in

which the specific binding of ^3H -dexamethasone was measured in cell suspensions derived from primary cultures of either palatal or maxillary mesenchyme cells obtained from A/J or C57 embryos. Irrespective of the origin of the mesenchyme cells (that is palatal or maxillary), the amount of dexamethasone-receptor activity (cytoplasmic and nuclear) was consistently higher in the mesenchyme cells of A/J than in the corresponding C57 cells (\sim twofold). A/J or C57 mesenchyme cells derived from either the secondary palate or maxillary processes do not differ significantly in total protein per cell, in the distribution of protein in cytosol or nuclear fractions (Table 2), or in the amount of ^3H -dexamethasone which is distributed *in vitro* at 37°C between the cytoplasmic and nuclear fractions (33.1 compared with 39% in the nuclear fraction for A/J and C57 cells, respectively) in cultured or freshly trypsinised cells. Therefore, the difference in total amount of glucocorticoid-receptor proteins in A/J and C57 mesenchyme cells as measured by whole cell binding seems to be partially accounted for by an apparent difference in the amount of dexamethasone-receptor protein(s) in the cytosols of A/J and C57 cells (see also Table 3).

Although C57 mesenchyme cells possess on average a lower concentration of dexamethasone-receptor proteins on a per cell basis (7,200 sites per cell compared with 16,300 sites per cell for A/J, calculated from the cell numbers), the apparent K_D for dexamethasone of the C57 receptors is higher, $\sim 7.9 \times 10^{-8} \text{ M}$, than the corresponding affinity of the receptors in the A/J cells for dexamethasone, $\sim 1.7 \times 10^{-8} \text{ M}$. The reason for these differences is not entirely apparent although a similar difference in the affinity constant for dexamethasone and quantitative differences in either the number or distribution of cytoplasmic and/or nuclear glucocorticoid receptors have been reported in cultured murine lymphosarcoma and L929 fibroblast cells between steroid-sensitive and resistant clones^{13,17–19}.

To determine whether these quantitative differences in dexamethasone-receptor activity between cultured A/J and C57 mesenchyme cells are due to alterations in the cells' ability to respond differentially in culture to certain growth factors which could alter the cell content of receptor proteins²⁰, dexamethasone-binding activity was measured in cytosol fractions prepared from the maxillary processes of 14-d-old embryos (Table 3). Although the absolute amount of ^3H -dexamethasone bound per mg cytosol protein in both A/J and C57 maxillary cytosols was six times less than the amount of ^3H -dexamethasone which was bound per mg total cell protein using whole cell suspensions (average 315 fmol of bound ^3H -dexamethasone per mg cell protein and 150 fmol per mg cell protein for cultured A/J and C57 cells, respectively), the dexamethasone-receptor activity was twice as high in the A/J maxillary cytosols (54.5 fmol bound ^3H -dexamethasone per mg cytosol protein) than in the C57 maxillary cytosols (23 fmol bound per mg cytosol protein). But the K_D for dexamethasone of the A/J cytosol receptor(s) ($\sim 2.9 \times 10^{-8} \text{ M}$) and for the C57 cytosol receptor(s) ($\sim 8.9 \times 10^{-8} \text{ M}$) were similar to those values estimated by using suspensions of cultured mesenchyme cells. Likewise, when freshly dissociated (trypsinised) A/J maxillary mesenchyme cells are labelled *in vitro*, they bind approximately three times as much dexamethasone (54 fmol bound ^3H -dexamethasone per mg cell protein) than the corresponding C57 mesenchyme cells (18 fmol bound ^3H -dexamethasone per mg cell protein). Therefore, the apparent difference in the amount of dexamethasone-receptor protein(s) between A/J and C57 mesenchyme cells is evident in cells which have either been freshly dissociated or grown in culture for several days. These results suggest that there is little or no loss of this phenotypic trait (receptor concentration) in primary cultures of mesenchyme cells.

These results demonstrate that mouse mesenchyme cells derived from either the secondary palate or maxillary pro-

Table 1 Characteristics of dexamethasone receptors in cultured mouse facial mesenchyme cells

Mesenchyme cells	A/J	Strain	C57BL/6J
Secondary palate	$N = 3$ $K_D = 1.3 \times 10^{-8} \text{ M} (\pm 0.28)$ $r = 420 \text{ fmol per mg cell protein } (\pm 150)$ $= 34 \text{ fmol per } 10^6 \text{ cells}$ $\sim 20,500 \text{ sites per cell}$ $P = 76.4 \text{ } \mu\text{g per } 10^6 \text{ cells } (\pm 27.2)$	$N = 4$ $K_D = 6.4 \times 10^{-8} \text{ M} (\pm 1.6)$ $r = 188 \text{ fmol per mg cell protein } (\pm 80)$ $= 14 \text{ fmol per } 10^6 \text{ cells}$ $\sim 8,400 \text{ sites per cell}$ $P = 78.4 \text{ } \mu\text{g per } 10^6 \text{ cells } (\pm 13.4)$	$N = 4$ $K_D = 9.3 \times 10^{-8} \text{ M} (\pm 0.38)$ $r = 110 \text{ fmol per mg cell protein } (\pm 30)$ $= 10 \text{ fmol per } 10^6 \text{ cells}$ $\sim 6,000 \text{ sites per cell}$ $P = 90 \text{ } \mu\text{g per } 10^6 \text{ cells } (\pm 10.7)$
Maxillary processes	$N = 4$ $K_D = 2.0 \times 10^{-8} \text{ M} (\pm 1.3)$ $r = 210 \text{ fmol per mg cell protein } (\pm 75)$ $= 20 \text{ fmol per } 10^6 \text{ cells}$ $\sim 12,000 \text{ sites per cell}$ $P = 90 \text{ } \mu\text{g per } 10^6 \text{ cells } (\pm 10.6)$	$N = 4$ $K_D = 7.9 \times 10^{-8} \text{ M} (\pm 0.38)$ $r = 150 \text{ fmol per mg cell protein } (\pm 40)$ $= 12 \text{ fmol per } 10^6 \text{ cells}$ $\sim 7,200 \text{ sites per cell}$ $P = 90 \text{ } \mu\text{g per } 10^6 \text{ cells } (\pm 10.7)$	$N = 8$ $K_D = 7.9 \times 10^{-8} \text{ M} (\pm 0.38)$ $r = 150 \text{ fmol per mg cell protein } (\pm 40)$ $= 12 \text{ fmol per } 10^6 \text{ cells}$ $\sim 7,200 \text{ sites per cell}$ $P = 90 \text{ } \mu\text{g per } 10^6 \text{ cells } (\pm 10.7)$
Average	$N = 7$ $K_D = 1.7 \times 10^{-8} \text{ M} (\pm 0.7)$ $r = 315 \text{ fmol per mg cell protein } (\pm 100)$ $= 27 \text{ fmol per } 10^6 \text{ cells}$ $\sim 16,300 \text{ sites per cell}$	$N = 8$ $K_D = 7.9 \times 10^{-8} \text{ M} (\pm 0.38)$ $r = 150 \text{ fmol per mg cell protein } (\pm 40)$ $= 12 \text{ fmol per } 10^6 \text{ cells}$ $\sim 7,200 \text{ sites per cell}$	$N = 8$ $K_D = 7.9 \times 10^{-8} \text{ M} (\pm 0.38)$ $r = 150 \text{ fmol per mg cell protein } (\pm 40)$ $= 12 \text{ fmol per } 10^6 \text{ cells}$ $\sim 7,200 \text{ sites per cell}$

Cumulative data from several different experiments (N , number of experiments) \pm s.e.m. showing the specific binding of ^3H -dexamethasone to receptors in whole cell suspensions. Data were analysed by the method of Scatchard¹⁶ to calculate the concentration of receptor-steroid complex (r) in equilibrium conditions and the affinity of the receptors for dexamethasone, dissociation constant (K_D). The protein content (P) was determined by the method of Lowry¹⁵. The number of sites per cell was calculated from the cell number, which was determined using a Coulter counter.

cesses possess cytoplasmic dexamethasone-binding proteins of high affinity and limited capacity. Furthermore, facial mesenchyme cells obtained from day 14 A/J embryos have on average approximately double the amount of receptor proteins than do mesenchyme cells obtained from C57 embryos at equivalent stages. This biochemical difference might partially account for the variations observed *in vivo* in the ability of these two strains of mice to respond differentially to the teratogenic effect of glucocorticoids.

The cytoplasmic dexamethasone receptors from A/J and C57 mesenchyme cells have biological activity in that cell suspensions labelled *in vitro* with ^3H -dexamethasone accumulate specifically bound radioactive steroid in their nuclei, presumably as a result of nuclear translocation¹⁴. The number of dexamethasone-receptor sites per cell as well as the percentage of ^3H -dexamethasone which is trans-

Table 2 Distribution of ^3H -dexamethasone binding in cytosol and nuclear fractions of mouse maxillary mesenchyme cells

Strain	Receptor activity Cytosol fraction	Nuclear fraction	% Nuclear transfer
A/J	97.7*	20.6	17.4(39.1)
	16†	15.2	48.7(34.3)
			Average: 33.1(36.7)
C57BL/6J	160*	103	39.2(33.1)
	33†	20.9	38.8(31.0)
			Average: 39 (32.1)

Whole cell suspensions from either freshly dissociated maxillary cells ($\sim 20 \times 10^6$ cells)* or primary mesenchyme cultures ($\sim 4 \times 10^6$ cells)† were incubated with $10 \text{ nM } ^3\text{H}$ -dexamethasone in the absence or presence of unlabelled dexamethasone (10^{-5} M) at 37°C for 40 min. The cells were then centrifuged, washed in ice-cold PBS, recentrifuged and resuspended in 1.0 ml of hypotonic buffer, composed of 10 mM Tris-HCl , $\text{pH } 8.0$, 1 mM MgCl_2 and 2 mM CaCl_2 , and lysed by freeze-thawing. The lysates were separated into crude nuclear pellets and crude cytosol supernatants after centrifugation ($200g$, for 5 min at 4°C). Aliquots of the latter were taken to assess the specific binding of ^3H -dexamethasone to cytosol fractions and to determine the protein content. The nuclear pellets were resuspended in 40 ml of ice-cold hypotonic buffer and centrifuged. The pellets were dissolved in 0.5 ml of distilled water and aliquots were taken to assess the specific binding of ^3H -dexamethasone in the nuclear fractions and to determine the protein contents. Receptor activity is expressed as fmol of specifically bound ^3H -dexamethasone per mg of cytosol or nuclear proteins. The percentage of nuclear transfer of the dexamethasone-receptor complex was determined according to Croce *et al.*²³ using the following formula:

$$\% \text{ nuclear transfer} = \frac{\text{fmol per mg nuclear proteins}}{\text{fmol per mg nuclear proteins} + \text{fmol per mg cytosol proteins}} \times 100$$

Values in parentheses represent the percentage of total cellular protein in the nuclear fractions.

Table 3 Characteristics of dexamethasone receptors in cytosols of maxillary processes

Strain	A/J	C57BL/6J
$N = 2$	$K_D = 2.9 \times 10^{-8} \text{ M}$ (± 2.2) $r = 54.5 \text{ fmol per mg}$ cytosol protein (± 1.5)	$N = 3$ $K_D = 8.9 \times 10^{-8} \text{ M}$ (± 2.6) $r = 23 \text{ fmol per mg}$ cytosol protein (± 6.7)

Pooled maxillary processes obtained from day 14 embryos were homogenised in 2.0 ml of buffer composed of 10 mM Tris-HCl , $\text{pH } 7.4$, 1.5 mM EDTA and $0.5 \text{ mM dithiothreitol}$ in a Kontes homogeniser (20–25 strokes) at 4°C . After homogenisation, sucrose was added to a final concentration of 0.5 M . Homogenates were centrifuged at $100,000g$ for 60 min at 4°C . Aliquots of the supernatants (cytosol fraction) were incubated at 4°C for 2 h with $1\text{--}100 \text{ nM } ^3\text{H}$ -dexamethasone in the absence or presence of an excess of unlabelled dexamethasone (10^{-5} M) to correct for nonspecific binding²¹. A suspension of dextran (0.5%, dextran T-70, Sigma) coated charcoal (5.0%, activated charcoal, Sigma) was then added to each assay to separate free from protein-bound steroid. The tubes were centrifuged at $1,500g$ for 15 min at 4°C , and aliquots of the supernatants were taken to assess the specific binding of ^3H -dexamethasone to cytosol proteins. The values in the table represent the average data obtained from Scatchard plots for each experiment (N , number of experiments) \pm s.e.m.

ferred into the nucleus in A/J and C57 is in agreement with the values reported for other steroid-responsive cells in culture^{18,21,22}. The presence of functional glucocorticoid receptors in both the palatal and surrounding facial mesenchyme cells favours the likelihood that these cells have the capacity to respond biologically to physiological levels of glucocorticoids. In fact, unmetabolised cortisol has been localised in the cells of the foetal maxilla of day 12 A/J embryos after *in vivo* administration of ^3H -cortisol²³.

Glucocorticoids are known to inhibit the growth of primary cultures of rat, mouse and chick embryonic fibroblasts^{24–26} as well as the multiplication of established mouse fibroblast lines such as L929 (ref. 27). Cortisone can also inhibit *in vivo* the proliferation of mesenchyme cells in the developing mouse secondary palate in A/J and H-Velaz mice^{28,29}. Experiments are in progress to determine whether the growth of primary cultures of mouse facial mesenchyme cells is altered *in vitro* by glucocorticoids.

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Reactivity of normal and brachypod mouse limb mesenchymal cells with con A

SINCE plant lectins, such as concanavalin A (con A) and wheat germ agglutinin (WGA), bind to specific carbohydrate residues¹, they have been used to investigate changes in plasma membranes during the course of normal development by monitoring lectin-induced agglutinability²⁻⁴, the binding affinities of carbohydrate moieties of cell-surface components for radioactively-labelled lectins^{3,5,6}, and the distribution of binding sites using fluorescently conjugated lectins⁷. It has been proposed that agglutinability with con A and WGA varies with the stage of differentiation of a

particular cell type^{1,2,5,6,8}. It seems reasonable to assume, therefore, that modifications of cell-surface structure, such as changes in carbohydrate components or in their distribution on or in the plasma membranes, could result in the manifestation of an aberrant phenotype. Support for this notion comes from studies in which a reduced affinity for insulin and various plant lectins by liver cell plasma membranes was observed in young, hyperglycaemic mice⁹. Several studies on hereditary skeletal disorders¹⁰⁻¹² including brachypodism (*bp*^H) in mice^{13,14} have suggested that these anomalies originate at a time when the surface-mediated events of cell recognition and sorting-out are resulting in the formation of prechondrogenic blastemata¹⁵. Since rudiments derived from the postaxial region of the hindlimbs are the most severely affected in brachypodism^{12,14} con A agglutination and binding experiments were carried out on mesenchyme cells from this region in both normal (+/+) and mutant (*bp*^H/*bp*^H) embryos during early limb development. The results presented here show that differences occur over the 3-d test period supporting the contention that plasma membrane surfaces change during developments^{1-6,8}. These developmental modifications are, however, temporally different between the two genotypically different cells which may be related to the ultimate manifestation of the abnormal phenotype.

Postaxial mesenchyme cells of 11-d stage embryos of both genotypes exhibited a similar degree of agglutination (Table 1). Cells from 12-d stage normal embryos, however, showed a significant reduction in agglutination, whereas agglutination of the brachypod cells remained essentially unchanged. It is during this period that myogenic and chondrogenic processes commence in normal mouse limb development²². The decreased agglutination exhibited by 12-d stage normal cells is in agreement with the contention that, as differentiation proceeds, con A and WGA agglutinability decreases^{1,8}.

A reduction similar to that observed in normal cells between the 11- and 12-d stages was not found until 24 h later

Table 1 Comparison of con A binding and agglutination of normal and brachypod limb mesoblast cells from 11-, 12-, and 13-d stage mouse embryos*

Stage and genotype	Percentage agglutination† (mean ± s.d.)	³ H-con A binding‡§ (c.p.m. / mg protein × 10 ⁻³)	Specific activity <i>bp</i> ^H / <i>bp</i> ^H / Specific activity +/+	Surface area (mm ²) (mean ± s.d.)
11-d				
+/+	45.4 ± 9.3	440		0.118 ± 0.030
<i>bp</i> ^H / <i>bp</i> ^H	40.9 ± 12.5	470	1.07	0.112 ± 0.029
12-d				
+/+	17.9 ± 10.2	210		0.123 ± 0.036
<i>bp</i> ^H / <i>bp</i> ^H	44.7 ± 13.7¶	540	2.57	0.118 ± 0.032
+/+ (trypsinised)	54.5 ± 4.7	400		
<i>bp</i> ^H / <i>bp</i> ^H (trypsinised)	56.2 ± 9.1	390	0.98	
13-d				
+/+	6.2 ± 2.7	390		0.108 ± 0.030
<i>bp</i> ^H / <i>bp</i> ^H	17.6 ± 10.9	280	0.72	0.110 ± 0.036

* Mutant and normal mesoblast cells from the postaxial region of the hindlimbs of 11-, 12-, and 13-d stage embryos were isolated as described previously¹⁸. After a 30-min treatment with Ca²⁺ and Mg²⁺-free (CMF) Tyrode's solution, dissociation was carried out in phosphate-buffered saline (PBS)¹⁷ containing 10 µg ml⁻¹ DNase³. In certain experiments, CMF treatment was replaced by a 20-min treatment with 1.5% trypsin at room temperature. Both types of dissociation treatments yielded cell suspensions of greater than 92% viability as determined by Trypan blue exclusion. Viability remained the same at the end of the experiments. Binding and agglutination experiments were carried out in the following conditions: a 0.4-ml reaction mixture containing 150 µg ml⁻¹ con A (Sigma) and 5.5 × 10⁶ cells in PBS-DNase solution was incubated in Falcon multiwell culture dishes for 20 min at 4 °C with occasional, gentle swirling. Controls for each genotype included 0.1 M α-D-methylmannose (Sigma) to inhibit lectin-induced agglutination. Aliquots of each reaction mixture were placed in depression slides and the percentage of total cells agglutinated (two or more cells in clumps) was determined by counting single cells against agglutinated cells in a minimum of six fields of view¹⁷. Statistical significance of the differences was determined using a two-group comparison *t* test¹⁸. Tritiated con A (New England Nuclear, 65.2 Ci mmol⁻¹) diluted with cold con A to a concentration of 150 µg ml⁻¹ was used for the binding experiments. Labelled cells were collected as described by Noonan and Burger¹⁸ and solubilised in Protosol (New England Nuclear) before counting in Omnifluor-toluene scintillation cocktail with a Beckman LS-100 scintillation counter. Protein determinations were carried out using Duly and Grieve's modification of the Lowry procedure^{20,21}.

† Expressed as percentage agglutination over that found in controls.

‡ Expressed as c.p.m. × 10⁻³ above what was determined for the controls.

§ Average value for at least three determinations.

¶ CMF-treated, 12-d *bp*^H/*bp*^H significantly different from CMF-treated, 12-d +/+ but did not differ significantly from 12-d trypsinised +/+ or *bp*^H/*bp*^H (two-group comparison *t* test, *P* = 0.01).

in the mutant cells (Table 1). This delayed decrease in agglutination corresponds with the delay in the ability of mutant cells to produce matrix components and stain meta-chromatically in the intact limb^{13,14}, in monolayer cultures¹⁶, and in rotation-reaggregation cultures²². It has been shown in other developing systems that the decrease in con A-induced agglutination is not accompanied by a concomitant drop in the binding of the lectin^{1,5}, suggesting that the decrease in agglutinability is due to the inability of the binding sites to rearrange into a display conducive to agglutination¹. This does not, however, seem to apply to this system (Table 1). There is a decrease in the amount of con A bound by the +/+ cells between days 11 and 12, whereas the mutants remain essentially the same. Comparing the specific activities of con A bound to bp^H/bp^H and that of +/+ at day 11, both genotypes bind approximately the same amount of lectin. The decline in the binding of con A by the +/+ cells at day 12 results in an increase in the ratio of specific activities, suggesting that twice as many binding sites are in bp^H/bp^H cells than in +/+ cells of the same gestational age. At day 13 the amount of lectin bound to bp^H/bp^H cells again approaches the normal condition. It is unlikely that the variations in binding can be explained solely by changes in surface area since this remained essentially unchanged in the two genotypes over the 3 d tested (Table 1).

The question remains as to whether the decrease in binding exhibited by the +/+ cells at day 12 is the result of loss of sites or masking of sites. Studies with trypsinised cells of both genotypes at day 12 (Table 1) supports the latter contention since the amount of tritiated lectin bound is the same with both trypsinised cell types. Enzyme-treated +/+ cells also exhibit the same degree of agglutination as trypsinised and non-trypsinised bp^H/bp^H cells. This supports the once held notion that trypsinisation unmasks "cryptic" sites²⁴ and suggests that 12-d stage +/+ and bp^H/bp^H cells possess the same number of con A-binding sites.

It is not understood at this time what the relationship is between the degree of agglutination, con A-binding sites, and the effect of the bp^H mutation. Work is presently in progress to determine agglutination with other plant lectins (WGA, fucose-binding protein) as well as the distribution of their respective binding sites using fluorescent conjugated lectins. Also, investigations using phenotypically normal bp^H /+ offspring are being carried out to determine the degree of functionality of the bp^H allele in the heterozygous condition.

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Genetic differences in susceptibility of pancreatic β cells to virus-induced diabetes mellitus

INFECTION of certain inbred strains of mice with the M variant of encephalomyocarditis (EMC) virus produces β -cell damage and a diabetes-like syndrome^{1–4}. The development of this syndrome is genetically determined and susceptibility is inherited as an autosomal recessive trait^{5,6}. The genetic factors involved seem to act at the level of the β cells, and *in vitro* studies have shown that β cells from susceptible strains of mice are more permissive to EMC infection than β cells from resistant strains of mice⁷. We have investigated first, the capacity of EMC virus to replicate in β cells from F₁ and F₂ offspring of susceptible and resistant strains of mice, and second, the effect of the viral infection on the level of insulin and glucose in the blood.

Four-to-five-week-old mice susceptible (SWR/J) and resistant (C57BL/6J) to the development of EMC-induced diabetes^{5,6}, as well as the F₁ and F₂ offspring, were infected intraperitoneally with 5.0×10^3 plaque-forming units (PFUs) of the M variant of EMC virus. At different times after infection, the pancreas was removed and β cells were isolated from individual mice as before⁷. In brief, the pancreas was minced, treated with collagenase (3 mg ml⁻¹ for 15 min at 37°C) and approximately 100 islets were collected from each pancreas with a stereo microscope. The islet-containing material was again treated with collagenase (7 mg ml⁻¹ for 6 min at 37°C), filtered through sterile gauze to remove large aggregates, and then layered on a 13–36% preformed Ficoll density gradient. The heavy visible band containing approximately 6.0×10^4 cells was collected⁷. Staining with aldehyde-thionin or fluorescein isothiocyanate-labelled anti-insulin antibody showed that almost 85% of the cells were β cells. These cells were then washed, frozen and thawed three times, homogenised and assayed for infectious virus on confluent monolayers of secondary mouse embryo cells. The concentration of insulin in the plasma was measured by the double antibody radioimmunoassay technique⁸ using mouse insulin (Novo A/S, Copenhagen) as the standard. Blood glucose levels were determined enzymatically by the glucose oxidase method with *o*-dianisidine dihydrochloride as the reactive dye⁹.

Figure 1 shows the relationship between insulin and glucose levels in the plasma at different times after infection. Before infection, the mean blood glucose level was 147 ± 19 mg per 100 ml with none of the glucose values above 182 mg per 100 ml or below 103 mg per 100 ml. The mean insulin level was 3.0 ± 0.95 ng ml⁻¹ with none of the insulin values below 1.9 ng ml⁻¹ or above 5.7 ng ml⁻¹. Within 48 h after infection, almost two-thirds of the SWR/J mice had insulin and glucose values outside these ranges. At 7 d, and even more clearly at 14 d, many of these mice became hypoinsulinaemic and hyperglycaemic. In contrast to the SWR/J mice, the glucose and insulin values of the C57BL/6J and F₁ hybrids generally fell within the normal range. Occasionally, 2 d after infection, a small percentage of the F₁ hybrids showed signs of hyperinsulinaemia, but this generally was not followed by hypoinsulinaemia. Several F₂ mice also showed signs of hyperinsulinaemia and hypoglycaemia 2 d after infection, but in contrast to the F₁ hybrids, at 7 and 14 d they became segregated into two groups—one with glucose and insulin levels within the normal range and the other showing severe hypoinsulinaemia and hyperglycaemia.

The viral titre in β cells isolated from mice at different times after infection is illustrated in Fig. 2. Data from 290 individual mice show that the greatest difference between SWR/J and C57BL/6J

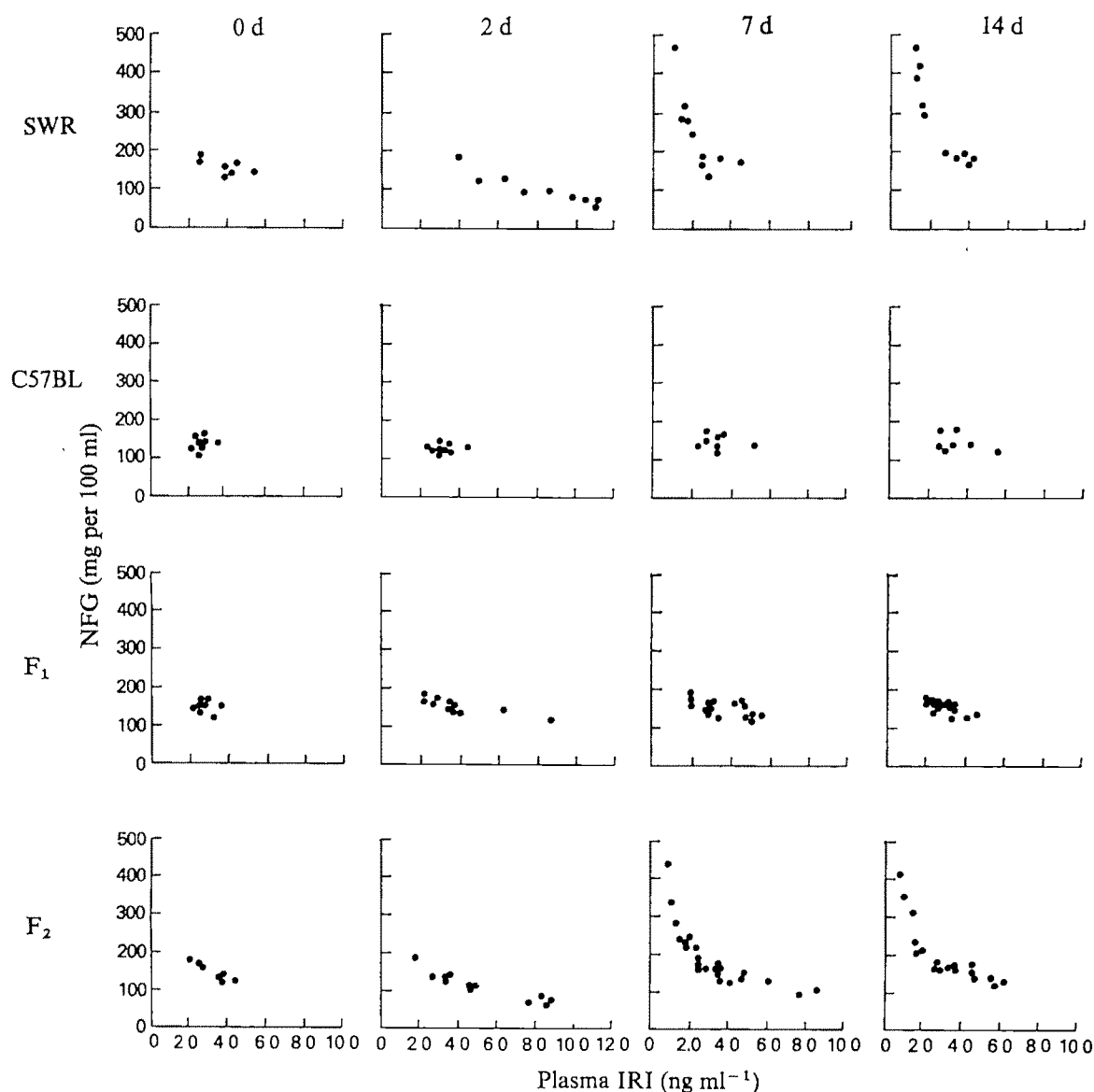


Fig. 1 Concentration of non-fasting glucose (NFG) and immunoreactive insulin (IRI) in the plasma of susceptible (SWR/J) and resistant (C57BL/6J) mice and their F_1 and F_2 offspring at different times after infection with EMC virus. Only male mice were tested and each point represents data from an individual animal.

occurred 48 h after infection and was more pronounced in males than females. At 48 h, up to 12 times more virus was recovered from β cells of SWR/J male mice ($2.6 \pm 2.8 \times 10^5$ PFUs) than C57BL/6J male mice ($2.1 \pm 0.7 \times 10^4$ PFUs), and analysis of variance revealed that the mean difference between the two groups was highly significant ($P < 0.005$). The mean virus titre in the β cells of the F_1 hybrids ($2.9 \pm 0.7 \times 10^4$ PFUs) was not significantly different from that of the resistant C57BL/6J mice ($P \geq 0.5$). The virus titre of individual F_2 animals, however, was spread out over a wider range and the standard deviation ($4.7 \pm 4.3 \times 10^4$ PFUs) was significantly different ($P < 0.005$) from that of the F_1 hybrids or the resistant C57BL/6J mice. The standard deviation of the F_2 females at 48 h also was significantly different ($P < 0.05$) from that of the female F_1 hybrids and the female C57BL/6J. Previous studies showed that male mice generally develop more severe hyperglycaemia than female mice after infection with EMC virus^{5,6}.

Figure 3 shows the relationship between viral titre and the levels of insulin and glucose in individual animals 48 h after infection. Although there were some exceptions, mice with the highest viral titre in the pancreas had the highest insulin and lowest glucose levels in the blood. Most of the SWR/J mice had viral titres

above 10^5 PFUs and their glucose and insulin values fell outside the normal range. In contrast, the resistant C57BL/6J and F_1 hybrid animals had viral titres below 10^5 PFUs and their glucose and insulin levels fell within the normal range. Animals in the F_2 generation segregated into two groups—the mice with viral titres above 10^5 PFUs had abnormal glucose and insulin values, while those with viral titres below 10^5 PFUs had glucose and insulin values within the normal range. Earlier studies had shown that the viral titres in the pancreas of susceptible mice was highest 2 d after infection and with the appearance of neutralising antibody at 3–4 d, the viral titre decreased^{5,7}.

We have shown that β cells from SWR/J mice are more susceptible to infection than β cells from C57BL/6J mice⁷. The experiments with individual mice reported here extend those observations, and show that the virus titre in β cells of F_1 hybrids was similar to that found in β cells of resistant C57BL/6J animals. Moreover, analysis of individual F_2 animals revealed two groups—one with viral titres approaching the susceptible SWR/J mice and the other with titres similar to that of the resistant C57BL/6J mice. Since the method of isolating β cells yielded preparations that were only about 85% pure⁷, the presence of virus

in the contaminating non- β -cell population might have obscured even greater differences in the actual viral titre in susceptible compared with resistant β cells. Nonetheless, our data show a correlation between viral replication and the subsequent development of clinical diabetes. Virus replicated best in β cells from SWR/J mice, and it was these animals that developed the most severe diabetes. Considerably less virus was found in β cells from C57BL/6J and F_1 hybrid mice, and these animals with high viral titres developed clinical diabetes. In contrast, the F_2 offspring segregated into groups with high and low viral titres; the animals with high viral titres were hyperinsulinaemic and hypoglycaemic at 2 d after infection due to acute β -cell damage and the rapid release of insulin. At about 7 d after infection these animals became hypoinsulinaemic and hyperglycaemic as a result of residual β -cell damage and the decrease in the insulin content of the pancreas^{1-4,7}. Because of the small size of the groups, the number of genes influencing susceptibility to virus-induced diabetes could not be analysed. Previous studies suggested that more than one gene was involved⁶.

Taken together, our studies suggest that EMC-induced hypoinsulinaemia and hyperglycaemia are secondary to genetically determined differences in the permissiveness of β cells to support viral replication. The demonstration of an association between particular HLA antigens (for example, B8 and BW15) and the frequency of juvenile diabetes supports the concept of a genetic predisposition to this disease¹⁰⁻¹⁴. The possibility that individuals

Fig. 2 Titre of virus in β cells from susceptible (SWR/J) and resistant (C57BL/6J) mice and their F_1 and F_2 offspring at different times after infection. Animals in the F_1 group represent both (SWR/J \times C57BL/6J) F_1 and (C57BL/6J \times SWR/J) F_1 . F_2 animals were bred from both (SWR/J \times C57BL/6J) F_1 and (C57BL/6J \times SWR/J) F_1 . Animals were infected intraperitoneally with 5.0×10^3 PFUs of EMC virus. The pancreas was removed, islets were collected and β cells were isolated by Ficoll density gradient centrifugation. The β cells were then homogenised and assayed for infectious virus on secondary mouse embryo cells. *a*, Male mice 24 h after infection; *b*, male mice 48 h after infection; *c*, male mice 72 h after infection; *d*, female mice 48 h after infection. Each point represents an individual animal and viral titre is expressed as PFUs per pancreas. Bars represent the geometric mean titre.

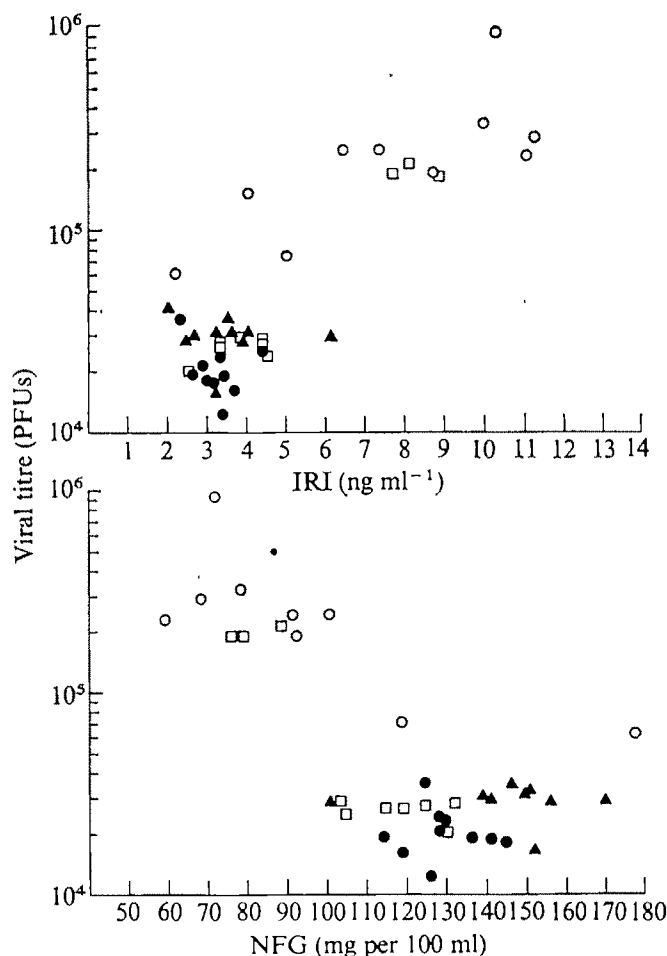
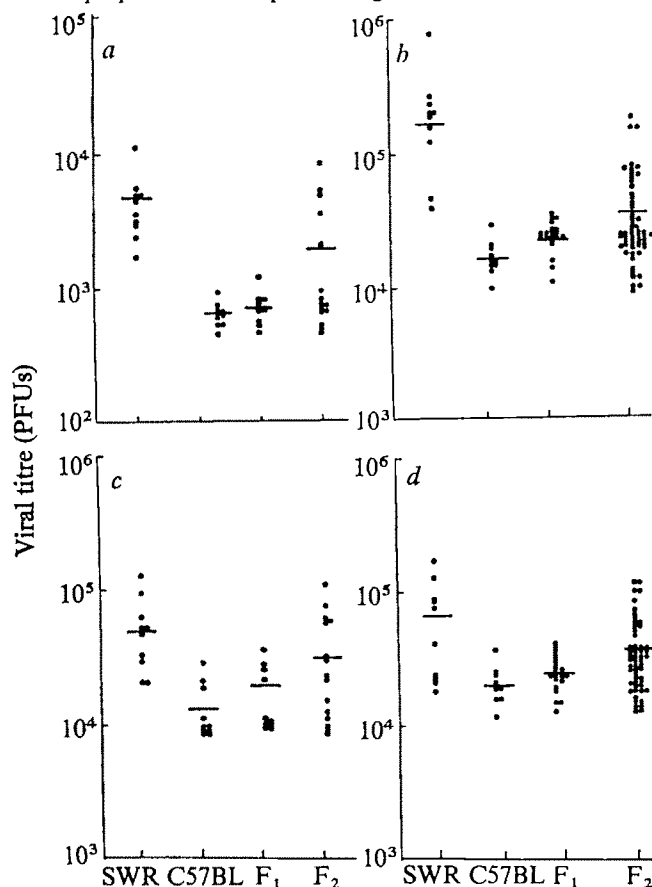


Fig. 3 Relationship between viral titre in β cells of the pancreas and level of insulin and glucose in the plasma of male mice 48 h after infection with EMC virus. See legend to Figs 1 and 2. \circ , SWR/J; \bullet , C57BL/6J; Δ , F_1 hybrids; \square , F_2 . Each point represents an individual animal.

of certain HLA types are more susceptible or respond differently to viral infections merits investigation.

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Activity of albumin conjugates of 5-fluorodeoxyuridine and cytosine arabinoside on poxviruses as a lysosomotropic approach to antiviral chemotherapy

THE therapeutic use of inhibitors of DNA synthesis as antiviral agents is hindered by their toxicity to dividing cells, which precludes the administration of effective doses. Consequently, although these drugs are very active in inhibiting virus replication *in vitro*, most of them are ineffective *in vivo*^{1,2}.

Albumin conjugates of two of these compounds, 5-fluorodeoxyuridine (FUdR)^{3,4} and cytosine arabinoside (ara-C) (G. B.-B. *et al.*, unpublished), release inhibiting substances in active form after penetration into the cells. This finding suggests a possible chemotherapeutic approach to attacking those DNA viruses which replicate in cells with a high protein uptake, that is, cells of the macrophage system⁵. After administration of such conjugates *in vivo* the inhibitors should be selectively concentrated in macrophages which are resistant to these drugs because they do not divide⁵. An attempt to concentrate in macrophages FUdR and ara-C by using their protein conjugates was suggested by the observation that the toxins amanitin and phalloidin after conjugation to albumin, change their original target selectively damage cells endowed with a high pinocytotic activity⁶⁻⁸.

Particularly suitable candidates to test this model of anti-

viral chemotherapy are poxviruses whose infectious cycle *in vivo* begins in macrophages⁹. We have studied the action of albumin conjugates of FUdR and ara-C: (1) on the replication *in vitro* of *Vaccinia* and *Ectromelia* viruses in cultures of L-929 cells and of mouse peritoneal macrophages; (2) on the growth of *Ectromelia* virus in mouse liver. *Ectromelia* virus, the agent of mousepox, is a poxvirus which, when injected intravenously into mice, is ingested mainly by the Kupffer cells in the liver where it starts replication. Then it infects neighbouring hepatic cells which in turn infect more hepatic cells after each cycle of growth. The resulting liver necrosis causes the death of mice^{11,12}.

Ectromelia virus (Hampstead mouse strain), grown in L-929 cells, was purified by zonal centrifugation in sucrose gradients according to Joklik¹³ and to Planterose *et al.*¹⁴ with slight modifications. The intravenous LD₅₀ of the purified virus for Swiss male mice of 28–30 g was 6.7×10^2 plaque forming units per g body weight. The *Vaccinia* virus used was from crude stocks produced in L-929 cells. The preparation of the FUdR-albumin conjugate (FUdR-RSA I) was carried out as described previously⁴ by coupling FUdR to crystalline rabbit serum albumin (RSA) by means of 1-ethyl-3-(dimethyl-aminopropyl)carbodiimide (EDCI) (Fluka). Ara-C-albumin conjugate (ara-C-RSA) was prepared by using the same concentration of reactants and by following the same procedure used for FUdR-RSA I. The molar ratios of FUdR and ara-C to RSA in the conjugates, determined in separate preparations by using ³H-FUdR and ³H-ara-C (Radiochemical Centre), were found to be 30 and 18 respectively. Sodium dodecyl sulphate (SDS)-polyacryla-

Table 1 Effect *in vitro* of free and conjugated FUdR and ara-C on *Ectromelia* and *Vaccinia* virus replication

Experiment	Compounds	$\mu\text{g ml}^{-1}$	Virus yield (log ₁₀ PFU ml ⁻¹)	
			<i>Ectromelia</i>	<i>Vaccinia</i>
1	Control		2.49	4.26
	FUdR	2.5	1.38 (45)†	1.72 (60)†
	FUdR-RSA I	25 (2.6)*	1.06 (57)	3.48 (19)
	FUdR-RSA I	50 (5.3)	0.80 (68)	1.36 (68)
2	Control		2.45	4.31
	FUdR	2.5	0.56 (77)	1.96 (55)
	FUdR-RSA II	25	1.23 (50)	2.74 (37)
	FUdR-RSA II	50	0.38 (84)	1.60 (63)
	EDCI-RSA	50	2.65 (0)	4.38 (0)
3	Control		2.29	4.22
	ara-C	2.5	0 (100)	0 (100)
	ara-C-RSA	25 (1.5)	1.58 (31)	2.13 (50)
	ara-C-RSA	50 (3.1)	0.38 (83)	1.42 (66)
4	Control		—	3.81
	FUdR-RSA I	50 (5.3)	—	1.39 (64)
	+ thymidine	100	—	3.91 (0)
	FUdR-RSA II	50	—	1.51 (60)
	+ thymidine	100	—	3.59 (6)
	ara-C-RSA	50 (3.1)	—	1.36 (64)
	+ deoxycytidine	50	—	2.69 (29)
	ara-C-RSA	50 (3.1)	—	
	+ deoxycytidine	500	—	2.99 (22)
5	Control		1.25	
	FUdR	7	0.25 (80)	
	FUdR-RSA I	50 (5.3)	0.38 (70)	
	FUdR-RSA II	100	0.87 (30)	

Cells were infected with *Ectromelia* or *Vaccinia* virus at the input multiplicity of 1 PFU per cell. Virus titres were determined by plaque counting in L-929 cells according to Blanden²¹ with an overlay medium containing 0.5% methylcellulose. Virus yields were obtained by subtracting the amount of virus adsorbed to cells from the virus titre at the end of the experiment. Mouse peritoneal macrophages were collected and cultured as described previously⁷. Experiments 1–4 were performed in L-929 cells, experiment 5 in mouse peritoneal macrophages.

*The amounts (μg) of inhibitors contained in the conjugates are shown in parentheses.

†Number in parentheses is the percentage of inhibition.

mide gel electrophoresis showed that only a small portion of the conjugates had a molecular weight corresponding to the monomeric form of albumin. The bulk of the conjugates was made up by molecular species corresponding to high molecular weight polymers of albumin which were formed in the presence of ECDI¹⁸. Since in ECDI conjugation stable covalent bonds are also formed between albumin and the urea derivative of carbodiimide¹⁸, RSA was allowed to interact with ECDI in the absence of FUDR or ara-C so that we could evaluate the possible antiviral activity of this complex (ECDI-RSA). We also studied the antiviral activity of another FUDR-RSA conjugate prepared by Dr de Vries in the laboratory of Professor Th. Wieland (Heidelberg). This conjugate (FUDR-RSA II) was obtained by coupling the hydroxysuccinimide ester of succinylated FUDR to RSA. This conjugation produces neither side reactions nor polymerisation of albumin¹⁶ and consequently avoids the heavy changes of protein molecules which follow carbodiimide coupling reactions. The molar ratio of FUDR to RSA in this conjugate was not known.

Table 1 shows that free and conjugated FUDR and ara-C inhibit the replication of *Ectromelia* and *Vaccinia* viruses *in vitro* after infection of L-929 cells or mouse peritoneal macrophages. *Ectromelia* virus yields in mouse peritoneal macrophages are lower than those in L-929 cells since virus replication is restricted in the former cells¹⁷. FUDR-RSA I and II are equally active *in vitro* on both viruses; ECDI-RSA has no effect. Thymidine and deoxycytidine, which are known respectively to remove totally or partially the effects of FUDR and ara-C¹⁸, counteract the antiviral activity of FUDR-RSA and of ara-C-RSA, indicating that the activity of the conjugates is due to the FUDR and ara-C moieties. Since albumin after penetration into the cells by endocytosis is broken down by lysosomal enzymes¹⁹, it is likely that the conjugates exert their antiviral activity after digestion of the protein moiety and release of the drugs, thus behaving as lysosomotropic agents^{20,21}. The assumption that the bonds linking FUDR and ara-C to RSA are broken after penetration of the conjugates into the cells is supported by the evidence that these bonds involve the primary hydroxyl

groups of FUDR and ara-C²² which must be phosphorylated so that the drugs can block DNA synthesis¹⁸.

The effects of FUDR-RSA and ara-C-RSA *in vivo* have been evaluated according to two criteria: (1) inhibition of virus production in liver; (2) survival time and number of survivors. The results of the experiments on virus production in mouse liver are given in Table 2. The compounds were injected intravenously. FUDR-RSA I, FUDR and ara-C were given at doses which, with the time schedule followed, corresponded to 1/3, 1/2 and 1/3 of the respective LD₅₀. Ara-C-RSA administered to normal mice with the schedule of experiment 3 but in doses three times higher did not kill any of the injected animals. Experiments 1-5 show that FUDR-RSA I and ara-C-RSA reduced significantly the virus yield in mouse liver whereas free FUDR and ara-C as well as ECDI-RSA were ineffective. Note that the active doses of the conjugates contained 19 and 15 times less FUDR and ara-C respectively compared with the amounts of free compounds used. FUDR-RSA II, which *in vitro* was as active as FUDR-RSA I was ineffective *in vitro* (experiment 4). The inactivity *in vivo* of FUDR-RSA II may depend on lack of aggregation and of profound protein changes, allowing the conjugate to escape a rapid uptake by macrophages²²⁻²³. In contrast, an aggregated and heavily modified albumin such as FUDR-RSA I is very quickly removed from the blood and taken up mainly by the Kupffer cells in the liver²²⁻²⁴. No reduction of virus titre by ara-C-RSA, determined in experiment 4, was found in the spleens. This result agrees with the observation that in mice the uptake of aggregated albumin is several times lower in spleen than in liver²⁵. Experiment 6 shows that ara-C-RSA is slightly active in reducing virus titre in the liver if its administration starts 12 h after infection, when the cycle of virus replication in Kupffer cells is completed^{11,12}. This finding demonstrates that the conjugates interfere mainly with the phase of virus infection which takes place in liver macrophages. This is supported by the results of experiment 7 showing that ara-C-RSA reduces the virus titre also if administered only within the first 12 h of infection. Table 3 shows the results of the survival experi-

Table 2 Effect *in vivo* of free and conjugated FUDR and ara-C on *Ectromelia* virus replication in mouse liver

Experiment	Compounds	Doses*	Virus yield (log ₁₀ PFU ml ⁻¹)
1	Controls FUDR-RSA I ECDI-RSA	100(0) 100(12) 100(12)	7.7 6.0 7.8
2	Controls FUDR FUDR-RSA I	200(0) 200(12) 150(12)	7.6±0.1 7.3±0.2 (NS) 6.3±0.2 P<0.001
3	Controls ara-C ara-C-RSA	70(0) 70(12) 150(12)	7.4±0.1 7.3±0.4 (NS) 5.6±0.8 P<0.005
4	Controls ara-C-RSA ara-C-RSA FUDR-RSA II	75(0) 75(12) 37(12) 150(12)	7.5±0.3 6.2±0.6 P<0.01 7.2±0.2 (NS) 7.4±0.4 (NS)
5	Controls ECDI-RSA	150(0) 150(12)	7.5±0.6 7.8±0.8 (NS)
6	Controls ara-C-RSA ara-C-RSA	150(0) 150(12) 150(24)	7.8±0.2 6.1±0.5 P<0.001 7.3±0.1 P<0.005
7	Controls ara-C-RSA ara-C-RSA	150(0) 150(0) 150(3)	7.8±0.5 6.4±0.8 P<0.01 6.6±0.4 P<0.005

Purified *Ectromelia* virus was injected intravenously into mice at the multiplicity of 2.4×10^6 PFU per g body weight corresponding to 360 times the LD₅₀. 48 h after infection livers were collected and homogenised in 5 ml of Hanks' balanced salt solution. Virus titres of clarified supernatants were determined in L-929 cells³¹. Five animals were used in each group. The results are given as the mean (\pm s.d.) of values obtained from livers of single animals, except in experiment 1 where livers from mice of each group were pooled before titration. The results were statistically evaluated by means of Student's *t* test and considered non significant (NS) when *P* was > 0.05.

*Doses are μ g g⁻¹ body weight; in parentheses, h after infection at which compounds were injected intravenously

Table 3 Effect of conjugates on survival of *Ectromelia* virus-infected mice

Experiment	Compounds	Doses*				Mean survival time (h)
1	Controls					72 ± 9
	FUDR-RSA I	150(0)	150(12)	50(24)	50(48)	65 ± 11 (NS)
	ara-C-RSA	150(0)	150(12)	50(24)	50(48)	94 ± 12 $P < 0.005$
No. survived/No. injected						
2	Controls					0/52
	ara-C	70(0)	70(12)	35(24)		0/7
	ara-C-RSA	150(0)	150(12)	50(24)		6/16 $P < 0.0005$
	ara-C-RSA	150(0)				5/24 $P < 0.01$
	ara-C-RSA	150(24)	150(36)	50(48)		0/12
	ECDI-RSA	150(0)	150(12)	50(24)		0/12

Experiment 1: mice were injected intravenously with the same amount of virus used in the experiments of Table 2. Twelve animals were used in each group. The results were evaluated statistically by means of Student's *t* test.

Experiment 2: mice were injected intravenously with 5.4×10^8 PFUs per g body weight, equivalent to 8 times the LD₅₀. Number of survivors was determined 21 d after infection. The results were evaluated statistically by means of the χ^2 analysis.

*For explanation of doses see Table 2.

ments. When mice were challenged with an amount of virus equivalent to 360 times the LD₅₀, ara-C-RSA increased significantly the mean survival time, whereas FUDR-RSA I was inactive. Since FUDR-RSA I reduces the virus titre *in vivo* to the same extent as ara-C-RSA, the different effect of the two conjugates on survival time is probably due to higher toxicity of the former. When mice were infected with eight times the LD₅₀ a significantly higher number of survivors was observed in the groups treated with ara-C-RSA than in the control group. Ara-C-RSA was active only if administered during the period of virus replication in Kupffer cells. Free ara-C and ECDI-RSA were ineffective.

In conclusion, albumin conjugates of FUDR and ara-C inhibit the growth of *Ectromelia* virus in mouse liver whereas the free inhibitors are ineffective. The finding that ara-C-RSA exerts its antiviral activity in liver macrophages indicates that ara-C is concentrated in these cells following the injection of the conjugate, providing experimental support to the lysosomotropic approach of virus chemotherapy. This observation also suggests that these or similar conjugates could be effective not only on viruses but also on other intracellular microorganisms growing in liver macrophages. In future experiments we will try to concentrate FUDR and ara-C in hepatocytes by use of a conjugate obtained by coupling these drugs to desialylated fetuin which is taken up specifically by parenchymal liver cells^{27,28} where it is digested by lysosomal enzymes²⁷. Such a conjugate should inhibit the replication of *Ectromelia* virus in hepatocytes. This line of research could have implications in the treatment of other hepatic viral infections such as human hepatitis B, whose agent has been localised in hepatocytes²⁹ and has been found to be a DNA virus³⁰.

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Experimental production of sheep pulmonary adenomatosis (Jaagsiekte)

SHEEP pulmonary adenomatosis (SPA) or Jaagsiekte is a contagious disease of unknown aetiology¹. A herpesvirus has been isolated from such tumours in Britain² and in Africa^{3,4} but attempts to produce adenomata with this virus have been unsuccessful (ref. 5 and W.B.M., G. Robinson, and K.W.A., in preparation). More recently, evidence has been presented that a type C virus with a density on a sucrose gradient of 1.5–1.20 g ml⁻¹, containing 60–70S RNA and RNA-directed DNA polymerase (reverse transcriptase) is present in the adenomatous tissue⁶. We wish to describe the production of SPA tumours in sheep experimentally infected with both agents.

Techniques⁷ similar to those of Perk *et al.*⁸, were used to confirm the presence of a reverse transcriptase-producing (RTP) agent in SPA tumour tissue from Scottish sheep. This enzyme was not detected in similar fractions from the lungs of unaffected sheep (Table 1).

A suspension of tumour from a natural case of SPA was subjected to equilibrium density centrifugation on a sucrose gradient. Fractions with densities of 1.15–1.17 g ml⁻¹ were centrifuged at 150,000g for 60 min at 4 °C and the pellet,

Table 1 DNA polymerase activity of fractions of tumour or unaffected lung

Sheep no.	Clinical state	Reagent added to standard reaction mixture	No addition	+Poly(A)-oligo(dT) ₁₀	+Actinomycin D
A525	Adenomatosis	60* (1)‡	930 (15)	70 (1.2)	
J2	Adenomatosis	150 (1)	370 (2.5)	33 (0.2)	
J4	Adenomatosis	40 (1)	330 (8.2)	26 (0.7)	
J13	Adenomatosis	110 (1)	800 (7.3)	115 (1.1)	
J14	Adenomatosis	153 (1)	750 (4.9)	133 (0.9)	
N1	Unaffected	120 (1)	155 (1.3)	10 (0.09)	
N15	Unaffected	117 (1)	173 (1.5)	47 (0.4)	
N16	Unaffected	170 (1)	313 (1.8)	93 (0.6)	

*Polymerase activity of 1.15–1.17 g ml⁻¹ fraction from sucrose density gradients of tumour or lung tissue suspensions, measured as increase in c.p.m. ³H-TTP incorporated into acid-insoluble material in 20 min using the methods outlined by Norval *et al.*⁷

‡Ratio of comparative activities are given in parentheses and the discriminatory effect of poly(A)-oligo(dT)₁₀ and actinomycin D on polymerase activity in the adenomata and healthy tissues are shown.

resuspended in 199 medium, was injected endobronchially into six Cheviot sheep. Herpesvirus isolated from an SPA tumour and passaged four times in embryo sheep kidney cells and once in sheep pulmonary macrophages (titre $\geq 10^{6.7}$ TCD₅₀ ml⁻¹) was similarly injected into four sheep. Both the herpesvirus and the density gradient fraction, containing the RTP agent, were injected into six more sheep. As controls, four sheep were given a supernate of tumour suspension and four, from the same flock, a supernatant prepared in an identical manner from apparently normal lung. To avoid cross infection, the groups of sheep were housed, as in previous transmission experiments, in separate loose boxes.

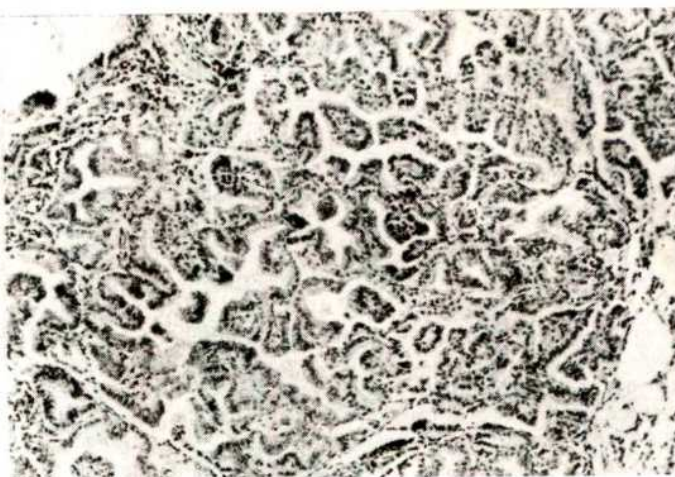
Results are shown in Table 2. One sheep, given the tumour supernatant, was clinically affected with SPA 6 months after inoculation. This diagnosis was confirmed histologically and all sheep were killed therefore 7–8 months after inoculation. A minimum of ten standard sites from the lungs of each sheep were examined histologically. In the group given the RTP agent alone, one sheep had two small (3–5-mm) adenomata and one other, histological evidence of adenomatous changes. Lesions in both sheep

Table 2 Evidence of pulmonary adenomata in the inoculated sheep

Material inoculated	No. in group	Macroscopic tumours	Histological adenomatous changes	Number with reverse transcriptase activity
Tumour supernatant	4	1	2	1
Herpesvirus and reverse transcriptase-producing agent	6	3	4	1
Reverse transcriptase-producing agent	6	1	2	0
Herpesvirus	6	0	0	0
Normal lung supernatant	4	0	0	0

Suspensions of finely minced tumour or lung tissue were prepared in Eagle's medium containing antibiotics and centrifuged at 12,000g for 30 min at 4 °C (inocula of "tumour or normal lung supernatant"). The supernate was recentrifuged at 120,000g for 90 min and the resulting pellet gently resuspended in 1 ml TNE buffer, layered on to a preformed gradient of 20–70% sucrose prepared in TNE buffer and centrifuged in a Beckman SW 40 rotor at 85,000g for 16 h at 4 °C. Fractions between densities of 1.15–1.17 g ml⁻¹ (the density of RNA tumour viruses) were pooled, diluted in TNE buffer and centrifuged at 150,000g for 60 min at 4 °C. The pellet obtained was suspended in 0.15 ml of TNE buffer and assayed for reverse transcriptase or further diluted for inoculation of sheep. Each inoculum was injected endobronchially in 8-ml volumes.

were minimal and present in four (13%) of a total of thirty-one sites examined. Of the sheep given both the RTP agent and the herpesvirus, three had definite macroscopic tumours and one was histologically positive (Fig. 1). No adenomatous lesions were found in any other sheep. All lungs were assayed for particles with reverse transcriptase activity and these were detected in lung tissue from two sheep (Table 2). Herpesvirus, which we have isolated from 24% of SPA tumours over the past 5 yr, was not recovered from any lung.

**Fig. 1** Focus of adenomatous change.

We can find no evidence to support the view that these tumours arose adventitiously or were caused by mycoplasma or other cultivable microorganisms. Since some sheep in both of the groups given the density fraction containing the RTP agent developed adenomatous lung lesions within 8 months of inoculation, it seems certain that this agent was involved in the production of these changes. Whether it alone is capable of initiating adenomatous changes or requires the assistance of the herpesvirus is conjectural and remains to be established. In the four affected sheep given both herpesvirus and RTP agent, lesions were extensive, and present in a mean of 63% of the areas of lung examined. This may indicate that the herpesvirus was involved in the production of these changes. The possibility that a small number of herpesvirus particles was present in the fraction containing the RTP agent cannot be dismissed completely and such contamination would simulate, to a lesser extent, the combined inoculum of herpesvirus and RTP agent. It may be that both viruses act synergistically to cause oncogenic transformation of the target cells, the type II alveolar cells or the cells of Clara in the bronchioles⁸, but the precise role of these viruses can only be elucidated by further experiments with purified virus.

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Specific inhibition of mitochondrial Ca^{2+} transport by antibodies directed to the Ca^{2+} -binding glycoprotein

WE have described a glycoprotein isolated from various mitochondria¹, which binds Ca^{2+} with a dissociation constant of the same order of magnitude as the K_m for Ca^{2+} transport^{2,3}. This and other observations, including the effects of known Ca^{2+} transport inhibitors^{4,5} suggest that the protein is involved in the carrier mechanism for the transport of calcium in mitochondria. We have concluded that even passive efflux of Ca^{2+} from mitochondria requires the glycoprotein to be associated with the membrane⁶, and we consider this to be strong evidence that the passage of Ca^{2+} across the mitochondrial membrane requires the mediation of a transport system including the glycoprotein. We have sought to confirm these conclusions by immunological methods.

Rabbits were immunised using 2 mg of purified ox liver glycoprotein isolated in our laboratory as before⁷. Antigen plus Freund's complete adjuvant was given in five injections into all four legs and the neck. The operation was repeated with Freund's incomplete adjuvant a month later. A secondary immune response was evoked by intravenous injection of 0.2-0.5 mg of glycoprotein, and at weekly intervals 40 ml blood samples were taken from the marginal vein of the ear. The serum γ -globulin fraction was obtained by Keckwick's method⁸. Lipoproteins were removed by acidification with 0.05 M acetate buffer, pH 5.6.

Fig. 1 Elution pattern from Affi-Gel 10-glycoprotein column of γ -globulin fraction from rabbit serum. A sample of 4 mg of pure ox liver Ca^{2+} -binding glycoprotein taken up in 25 ml of 0.1 M bicarbonate-HCl buffer, pH 7, was added in 1 g of Affi-Gel 10 (BioRad). The mixture was stirred at 0 °C for 24 h. The resin was packed in a short column and washed free of 260 nm-absorbing material. 10 ml of 1 M ethanolamine was passed through the column and equilibration was carried out using 50 mM triethanolamine-HCl buffer, pH 7.8 containing 100 mM KCl. The sample was added in the same buffer.

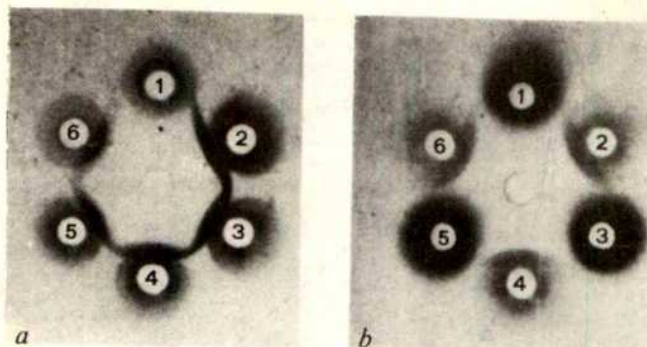
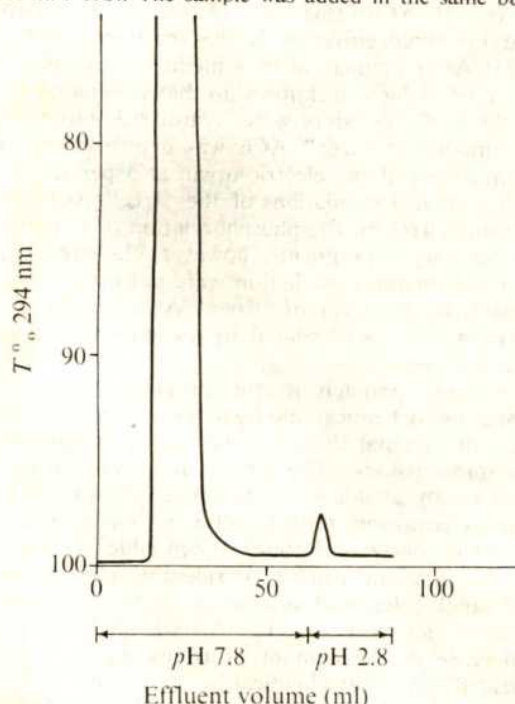


Fig. 2 Immunodiffusion patterns of γ -globulin fractions before (a) and after (b) affinity chromatography on Affi-Gel 10. See text for explanation.

Preliminary experiments with γ -globulin fractions, derived from the same animal before and after immunisation with the glycoprotein, had shown that γ globulins could to some extent inhibit Ca^{2+} transport in rat liver mitoplasts (mitochondria devoid of the outer membrane). To avoid non-specific effects, we purified the specific antibodies by affinity chromatography (Fig. 1). Only a small portion of the total protein was retained on the column and was eluted only when the pH was shifted from 7.8 to 2.8 (500 mM formic acid-ammonium formate). Since the limiting factor in this experiment is the amount of antigen coupled to the resin, the whole procedure was repeated four times. The last passage on the column yielded only negligible amounts of specific antibodies. We calculated that the overall purification was of the order of 150 times. Starting from about 1.5 g of serum protein, 10 mg of purified antibodies was obtained. Figure 2 shows the immunodiffusion patterns obtained by Ouchterlony's method⁹ by testing the γ -globulin fraction before and after immunisation with the glycoprotein. The central well (Fig. 2a) contained the isolated glycoprotein, well (1) contained the γ -globulin fraction before immunisation and all the other wells (2-6) contained γ -globulins obtained from the animal after secondary immunoresponses had been evoked at approximately 20-d intervals. A single antibody class was present in all samples. As a control (Fig. 2b) proteins after complete absorption on a column of Affi-Gel were tested against the glycoprotein. They did not contain appreciable amounts of antibodies (wells (1), (3) and (5)). Immunoprecipitate was present in wells (2), (4) and (6), where the γ -globulin fraction had been placed before affinity chromatography.

Figure 3 shows the inhibitory activity of the purified antibodies on Ca^{2+} transport by mitoplasts and intact mitochondria. Ca^{2+} transport by mitoplasts showed a hyperbolic dependence on antibody concentration, as if the antibodies were titrating the specific sites freely accessible to them. As expected, on the other hand, mitochondria were much less sensitive to added antibodies, and decayed linearly as antibody concentration increased. Half inhibition was attained at approximately 1.5 μg antibody protein per mg protein with mitoplasts. The same effect was obtained in mitochondria with an antibody concentration about five times greater. It could be argued that antibodies against a mito-

Table 1 Effect of specific antibodies to the Ca^{2+} -binding glycoprotein on Ca^{2+} transport, electron transport and respiratory control ratios in isolated rat liver mitoplasts

Antibody added (μg)	% Inhibition		
	Ca^{2+} transport	Electron transport	RC
5	45	0	0
20	70	17	10
40	84	37	19

A Clark oxygen electrode was used at 30 °C (ref. 13). The substrate succinate and reaction mixture were as described before¹⁰. RC, Respiratory control.

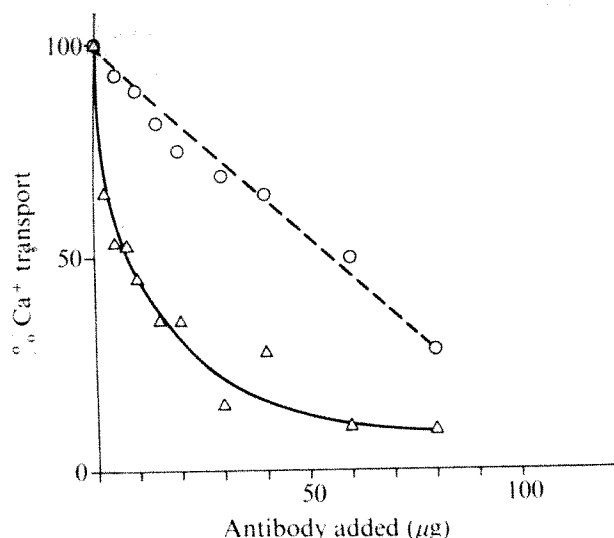


Fig. 3 Effect of antibodies against the glycoprotein on Ca^{2+} transport in rat liver mitochondria and mitoplasts. Mitochondria and mitoplasts were prepared as before¹⁰; Ca^{2+} transport was followed by the murexide method¹¹ in a mixture containing in 3 ml: 5 mg mitochondria or mitoplast protein (Biuret reaction of Gornall *et al.*¹²), 100 μM murexide, 70 mM sucrose, 220 mM mannitol, 2 mM HEPES, pH 7.4, 5 mM acetate, 1 mM phosphate buffer, pH 7.4, 15 μM rotenone, 165 μM Ca^{2+} , 5 mM succinate. A 540–507 was recorded at room temperature with a Phoenix dual wavelength recording spectrophotometer. ○, Intact mitochondria; △, mitoplasts.

chondrial membrane component exert an inhibitory effect by inhibiting either electron transport or the energy conservation mechanism or both. Table 1 compares the effect of antibodies on Ca^{2+} transport, electron transport and respiratory control ratio in mitoplasts. Clearly low concentrations of antibody can block 45% of Ca^{2+} transport without effect on other mitochondrial activities. Even at higher concentrations the effect on electron transport and on respiratory control ratio was definitely lower than that on the movement of the cation.

These data indicate that specific blocking of the calcium-binding glycoprotein inhibits active Ca^{2+} transport. It is not surprising that the effect is much more evident in mitoplasts in view of the barrier present in intact mitochondria to the interaction between glycoprotein and antibody. On the other hand, intact mitochondria would be expected to be sensitive to the antibodies, in spite of the impermeability of the outer membrane to proteins. Preincubation for 1 h is necessary to obtain an almost complete combination between antigen and antibodies. During this time it is reasonable to assume that proteins could diffuse slowly across the outer mitochondrial membrane. This process has to be linearly correlated with the antibody concentration outside the mitochondria, which would explain the linear inhibition of Ca^{2+} transport in mitochondria. These data indicate that the mitochondrial calcium-binding glycoprotein is a necessary ingredient in mitochondrial Ca^{2+} transport.

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Possible involvement of thiamine in acetylcholine release

ALTHOUGH it has long been thought that thiamine (vitamin B_1) and its phosphoric acid esters might be linked directly to excitation and propagation of nerve action potentials^{1,2}, the relationship has not been established firmly^{3,4}. We wish to report results which suggest that involvement with synaptic transmission rather than axonal conduction might explain why vitamin B_1 is required for nervous system activity. Experiments were performed on the electric organ of *Torpedo marmorata*, a richly innervated cholinergic tissue⁵. The results indicated that thiamine and its phosphoric esters are involved, either directly or indirectly, in the release of the neurotransmitter acetylcholine (ACh).

A first approach to the problem was made by comparing, in different conditions, ACh content with that of thiamine and thiamine phosphate esters of the electric organ (Table 1). Total thiamine content (thiamine plus esters) was found to range between 350 and 500 nmol g^{-1} of wet weight, with a rather large variation from one *Torpedo* to another. The relative distribution of thiamine, thiamine monophosphate (TMP) and thiamine diphosphate (TPP) was different in the following conditions. (1) When the tissue was incubated in physiological medium, most of the compound was found in the form of phosphate esters and the total content of ACh could be extracted without loss. (2) Homogenisation in the same medium before extraction resulted in significant hydrolysis of TPP into thiamine and a 45% loss of ACh; this part of the total transmitter destroyed by homogenisation is the fraction called "free ACh"⁶. (3) After incubation in a medium with high Mg^{2+} and low Ca^{2+} , which is known to block synaptic transmission, 33% of the esters were hydrolysed into thiamine and the amount of "free" ACh was decreased. (4) Continual stimulation of the electric organ at 5 per s, reported to induce sustained oscillations of the "free" ACh level^{7,8}, had the same effect on the phosphorylation of thiamine. In these preliminary experiments, however, the precise parameters of the thiamine oscillation were not established. We concluded that the level of "free" ACh in the electric organ was in some way related to the phosphorylation of thiamine.

In a second approach to the problem, electrophysiological and radiochemical methods were used to measure the effects of external thiamine and related compounds on synaptic transmissions. The first compound studied was oxythiamine, an analogue of thiamine known to inhibit the enzymes requiring TPP as cofactor but to have little or no effect on nerve excitation⁹. When added to the saline medium, oxythiamine caused considerable changes in the shape of single electrical discharges; both the amplitude and duration increased (Fig. 1). This was undoubtedly due to an increase in the amount of transmitter released, as demonstrated in radiochemical experiments (Fig. 2); samples treated with oxythiamine released more ACh than

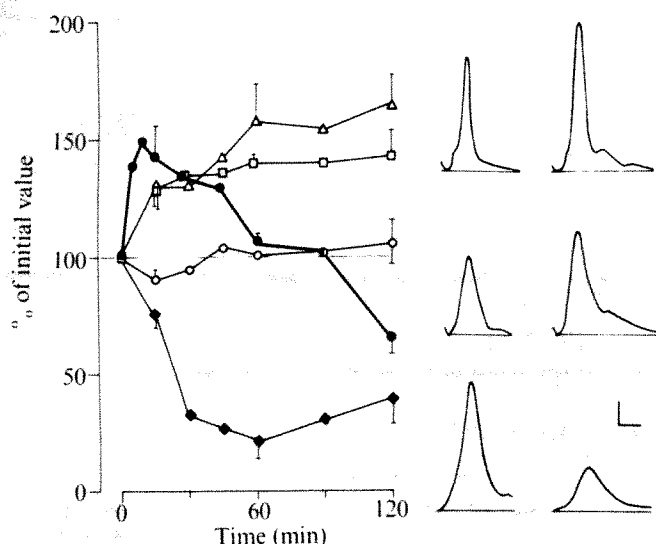


Fig. 1 Effects of external thiamine, oxythiamine and adenosine on the electrical discharge. Pieces of tissue containing one to three prisms were excised from the electric organ and their response to single shocks were tested at long time intervals. The prisms are made of 400–500 superposed electroplaques and, when submitted to nerve or field stimulation, they generate an electrical discharge which is the sum of a large number of synchronised postjunctional potentials^{5,7,8}. When incubated in normal physiological medium, the tissue produced, for several hours, constant responses to single stimulations. The right hand upper tracings in the figure show discharges recorded before and after a 60-min incubation in 5×10^{-3} M thiamine (calibration: 0.2 V, 2 ms). Middle records: effect of 10^{-4} M oxythiamine for 45 min (0.5 V, 2 ms). The effect of oxythiamine was related to its concentrations in the 10^{-5} – 10^{-3} M range. Lower records: 5×10^{-3} M adenosine for 60 min (0.2 V, 2 ms). In the graph, the surfaces of the discharge are expressed as percentage of their initial values and plotted as a function of time; s.e. is indicated only for crucial points. Δ , 5×10^{-3} M thiamine; \square , 10^{-4} M oxythiamine; \circ , controls; \bullet , 10^{-2} M thiamine; \blacklozenge , 5×10^{-3} M adenosine.

the controls with the most striking difference observed during the first 2 min of continual stimulation.

The same methods were then used to study adenosine, which has been reported to reduce "quantal release" at the neuromuscular junction¹⁰. The effect of this compound, unlike that of oxythiamine, was to reduce the electrical discharge and decrease the quantity of ACh released (Figs 1 and 2). Addition of 10^{-3} M adenosine to the medium could reverse the effect of 10^{-4} M oxythiamine in 15 min.

Experiments in which 10^{-3} M TPP was added to the medium, showed that this ester had no effect on synaptic transmission, probably because of its inability to penetrate cell membranes⁴. The effects of thiamine itself were a function of concentration and time (Fig. 1). At a concentration of 10^{-2} M, thiamine first caused an increase in

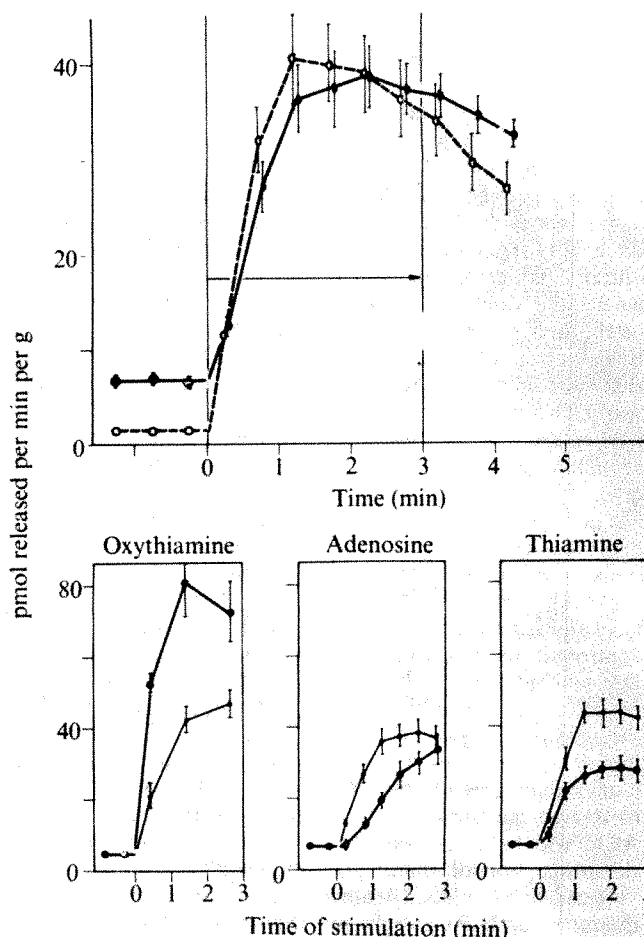


Fig. 2 Release of radioactive ACh from pieces of the *Torpedo* electric organ. The tissue was incubated for 4 h in physiological medium in the presence of ^{14}C -acetate (—, 132 d.p.m. μmol^{-1}) and ^3H -choline (---, 1,320 d.p.m. μmol^{-1}) at the same concentrations (8.4 μM). In this tissue, the two precursors are incorporated into ACh^{14,15}. The fragments were washed overnight and then submitted to continual stimulation at 10 per s for 3 min during superfusion at 1 ml min^{-1} . ^{14}C and ^3H radioactivities were counted in the superfusing solution and provided a sensitive and direct measurement of the ACh released during stimulation. At rest, the level of radioactive choline was constantly less than that of acetate, but, on stimulation, they increased in a ratio of 1:1. Lower graphs, Effects of 10^{-4} M, oxythiamine, 10^{-3} M adenosine and 5×10^{-3} M thiamine after 1 h of incubation. Only the ^{14}C values are shown here because they were not significantly different from the ^3H values. The fine lines indicate controls. In the oxythiamine experiment, ^3H -acetate and ^{14}C -choline were used. Adenosine (5×10^{-3} M) had the same effects as 10^{-3} M. In the presence of adenosine, the electrical discharge started at a lower level than in controls, but it increased during stimulation, an effect already described for ATP in this tissue¹⁶. Thiamine (10^{-3} M) increased slightly, but not significantly, the amount of ACh released.

Table 1 Comparison between the phosphorylation of thiamine and the level of "free" ACh in the electrogenic tissue of *Torpedo* in different conditions

Extraction after	Thiamine (%)	TMP (%)	TPP (%)	"Free" ACh (nmol g^{-1})
Incubation in the physiological medium (11)	28.6 ± 2.5	28.6 ± 5.5	42.8 ± 5.2	296 ± 29
Homogenisation in the physiological medium (8)	46.1 ± 6	28 ± 8	26 ± 5.7	0
Incubation in the medium with 5.3 mM Mg^{2+} and 0.01 mM Ca^{2+} (4)	62 ± 15	4 ± 4	34 ± 13	58 ± 27

Pieces of electric organ were excised under Tricain anaesthesia and placed in a physiological medium suitable for elasmobranch fish⁶. Thiamine and its phosphoric esters were extracted with 5% trichloroacetic acid, separated by high voltage electrophoresis and their concentration was determined by the fluorimetric procedure of Itokawa and Cooper¹². ACh was measured in the same extracts by a radiochemical assay¹³. The values of thiamine, TMP and TPP are expressed as percentage (\pm s.e.) of the total content of thiamine plus esters; this total was 458 ± 92 nmol g^{-1} wet weight and did not show significant variations between the three conditions. "Free" ACh was determined as the difference between the total ACh (708 ± 64 nmol g^{-1}) and the ACh remaining after homogenisation of the tissue⁶. Thus "free" ACh was equal to zero by definition in the second condition. Numbers of experiments are given in parentheses.

the electrical discharge but, in a second phase, the discharge decreased considerably, the effects of thiamine thus becoming similar to that of adenosine. At a lower concentration (5×10^{-7} M) of thiamine, amplification of the discharge lasted longer but after 1 h transmitter release had already diminished (Figs 1 and 2). This apparent discrepancy is due to the thickness (4–6 mm) of the pieces of tissue, through which thiamine requires time to diffuse⁶, since it is from the surface of the samples that the transmitter is collected, but it is chiefly at the core of the tissue where the discharge is generated. The effect of thiamine in the first phase differed from that of oxythiamine in that it was essentially the amplitude of the electrical discharge that was increased rather than the duration. This effect was transient, however, for in a second phase, thiamine, particularly at a high concentration, caused a sharp reduction of the discharge, related to the decrease in the quantity of ACh released.

The rather unexpected electrophysiological changes observed in this study are unlikely to be due to modifications of the rate of ACh hydrolysis by acetylcholinesterase, for the inhibitors of this enzyme are known to alter nerve-electroplaque transmission in a different way⁷. This was confirmed in biochemical tests which revealed that oxythiamine and adenosine, at the given concentrations, had no effect on the activity of cholinesterase extracted from the electric organ. Thiamine was also found not to inhibit the rate of hydrolysis except at a concentration higher than that of the ACh used as substrate. This might be due to the capacity of thiamine to form complexes with ACh, which have been found to be increasingly stable as the proportion of thiamine is increased¹¹.

It can be concluded from the observations of endogenous thiamine and ACh release, that the presynaptic nerve endings are the most probable site of thiamine involvement in synaptic transmission. It seems, moreover, that thiamine might even be directly implicated in transmitter release. A possible mechanism to explain its role would be as follows: thiamine might, in some way, bind to a fraction of "free" ACh, the pool of transmitter available for release⁶⁻⁸. This fraction, under the influence of Ca^{2+} , would then be released in the synaptic cleft, perhaps in a quantal manner. The release would be accompanied by a transfer of phosphate either to or from the thiamine and might occur at the presynaptic membrane, possibly with the involvement of synaptic vesicles. External oxythiamine as well as thiamine in the first phase, would cause an increase in the fraction of transmitter loaded and released, whereas adenosine or an excess of thiamine would cause a decrease.

Further investigations are needed to establish the validity of this theory as well as to determine whether thiamine is also involved in the release of other transmitter substances. Our results, however, suggest that the requirement of the vitamin B₁ by the nervous system can be better explained by its involvement in synaptic transmission rather than in axonal conduction.

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An inhibitory tripeptide from cat spinal cord

UNTIL quite recently, the possibility that naturally occurring peptides, with a few exceptions, such as substance P, might function to modify the excitability of neurones in the central nervous system (CNS) was considered rather remote. There is now increasing evidence to suggest that peptides have a greater range of actions and are more widely distributed than previously thought¹⁻⁶. In a search for physiologically active peptides in the CNS we have isolated a tripeptide from the dorsal columns of cat spinal cord, which has been partially characterised as His-(Gly, Lys). We have synthesised the two possible forms and tested their activity by iontophoretic application to single neurones in the spinal cord, the medial medulla oblongata and the dorsal column nuclei.

To extract peptides from central nervous tissue we used a technique which minimises degradative processes⁷. Lumbar segments L2–L5 of the spinal cord were exposed in barbiturate-anaesthetised cats and frozen *in situ* with liquid nitrogen. The frozen tissue was excised and allowed to thaw to 4 °C, when the dorsal columns were dissected out and homogenised in acetone: 1 M HCl (100:3 v/v) at –20 °C. The homogenate was stirred at 4 °C for 24 h. After centrifugation, the supernatant was lyophilised, suspended in a small volume of isopropanol–water (10:90 v/v), and applied to a Sephadex G-15 column. Peptides were detected in the column effluents by their absorption at 206 nm. One of the G-15 column fractions was run on a DEAE–Sephadex column to separate the peptides further and a fraction from this column was analysed by thin-layer chromatography.

Two separate methods were used to isolate individual peptides from this fraction. Dansylated peptides were chromatographed in two dimensions on polyamide plates; non-dansylated peptides were chromatographed on silica gel plates and located using fluorecamine. Fourteen components were detectable and one of them (that with the strongest fluorescence) had the R_f values shown in Table 1. This component, a discrete spot, was scraped from the polyamide plate. After hydrolysis, it was rerun on a second polyamide plate, when only dansyl-histidine could be detected, indicating that the N-terminal amino acid was histidine. The remainder of this second plate was eluted and dansylation of the concentrated eluate yielded only glycine and lysine on further chromatography. Since the elution time on Sephadex G-15 suggested a tripeptide structure, we concluded that the peptide was His-(Gly, Lys).

The two possible tripeptides His-Gly-Lys and His-Lys-Gly

Table 1 R_f values of the tripeptide His-(Gly, Lys) from cat spinal cord

	First dimension Solvent 1	Second dimension Solvent 2
Non-dansylated peptide (silica gel F254 plate)	0	47
Dansylated peptide (polyamide-6 plate)	Solvent 3 85	Solvent 4 5

The solvents were as follows: 1, butanol–acetic acid–water (4:1:1 by volume); 2, pyridine–acetic acid–water (10:6:3 by volume); 3, water–formic acid (200:3 v/v); 4, benzene–acetic acid (9:1 v/v).

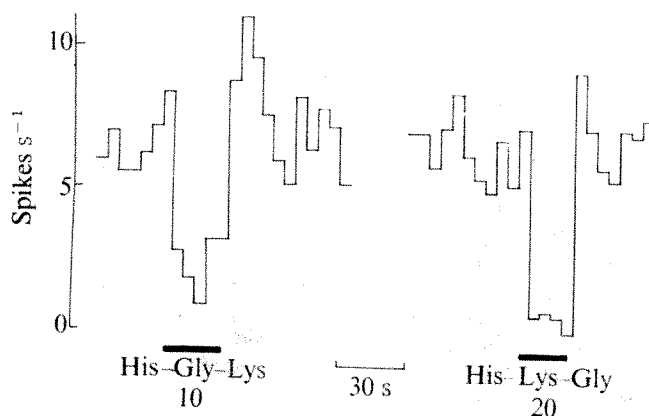


Fig. 1 The effects of iontophoretically applied His-Gly-Lys and His-Lys-Gly on the firing rate, induced by a continuous current of 4 nA of L-glutamate, of a neurone in the dorsal horn of the lumbar spinal cord. Na^+ (20 nA) had no effect. Applications are indicated by the horizontal bars; currents are shown in nA. Ordinate: Mean firing rate in impulses s^{-1} in successive 5-s epochs. Solutions of the peptides were made up to 0.05 M, pH 4.1–4.5.

were synthesised by conventional methods by two different routes. Both utilised mixed anhydrides or azides for coupling but differed in the type of protective groups used. In one set of syntheses, the carboxyl terminus was protected with benzyl, the sidechain of Lys and the α -amino group of His with benzyloxycarbonyl. The alternative scheme utilised *tert*-butyl for the carboxyl terminus and *tert*-butyloxycarbonyl for the Lys side-chain and His α -amino group. After removal of the protective groups by hydrogenolysis or protonolysis, respectively, the free peptides were purified by ion-exchange chromatography on carboxymethyl cellulose to yield homogeneous materials as shown by thin-layer chromatography and electrophoresis in several systems. The products of all four syntheses contained the correct ratio of amino acids after both acid hydrolysis and digestion with aminopeptidase M, indicating absence of racemisation during synthesis. H-His-Lys-Gly-OH triacetate had $[\alpha]_D^{26} = -12.3^\circ$ ($C = 1$, water) whereas H-His-Gly-Lys-OH triacetate had $[\alpha]_D^{26} = +5.6^\circ$ ($C = 0.8$, water).

The two synthetic peptides and the endogenous peptide had identical R_f values in the thin-layer chromatographic systems used and the same elution time on the Sephadex G-15 column. Consequently, the amino acid sequence of the natural peptide could not be assigned by direct chromatographic comparison with synthetic samples and will have to await the isolation of further material. Nevertheless, the characterisation of the isolated peptide as His-(Gly, Lys) is supported by the identical chromatographic behaviour of the synthetic and naturally occurring compounds.

Both synthetic tripeptides were applied by iontophoresis to single neurones in three regions of the CNS in unanaesthetised, decerebrate, decerebellate cats (mid-collicular decerebration was performed under halothane anaesthesia). Since the natural peptide was extracted from the dorsal columns, the responses of neurones in the dorsal column nuclei were examined first, and this was followed by application to cells in the spinal cord and the medial brainstem. The peptides were tested for effects on spontaneous activity or, when this was low or absent, on activity induced by the continuous application of the excitant L-glutamate from another barrel of the micropipette.

The dorsal column nuclei were located from surface landmarks and neurones were identified by their localised ipsilateral receptive fields in response to hair movement or joint movement or touch. Of 24 cells tested with His-Gly-Lys, 21 (12 in the cuneate nucleus and nine in the gracile nucleus) were unaffected by currents up to 150 nA, whereas three neurones in the gracile nucleus were inhibited at currents from 50–100 nA. His-Lys-Gly was tested on 15 cells; one in the gracile nucleus was

inhibited and the remainder in both dorsal column nuclei showed no response.

In the spinal cord (L3–L5), the peptides affected a much larger proportion of neurones. When applied with currents of 10–50 nA His-Gly-Lys inhibited (Fig. 1) 17 (40%) of 42 neurones, the remainder being unaffected. The positions of four cells inhibited by His-Gly-Lys were marked with pontamine blue, and all of these were in the dorsal horn; three seemed to be in lamina V and one was at the border of laminae VI and VII. His-Lys-Gly was tested on six cells, two of which were inhibited.

To test the peptides on cells in the medial brainstem, micropipettes were inserted through the floor of the IVth ventricle between 1.0 and 4.8 mm rostral to the obex and 1.0–1.5 mm lateral to the midline. As in the spinal cord, both peptides had depressant effects on some cells. His-Gly-Lys inhibited 30 (52%) of 58 units and His-Lys-Gly 11 (37%) of 30 units. Some of these neurones were identified as reticular by their response to somatosensory stimulation over a large area of body surface.

In all three regions of the CNS the reduction in firing rate was rapid in onset, beginning within the first second of the ejection period and reaching a plateau within 15–20 s (Fig. 1). At the end of the application the firing rate returned rapidly to its previous level. The degree of inhibition was related to the strength of iontophoretic current, and clear responses were sometimes seen with currents as low as 10 nA. No excitatory effects were seen. In most cases when similar currents were used to eject the two compounds, His-Gly-Lys produced a greater degree of inhibition than His-Lys-Gly. In all, His-Gly-Lys inhibited 50 of the 124 units examined (40%) whereas His-Lys-Gly inhibited only 14 of 51 units (27%). The former thus seems to be more potent than the latter, although a more accurate comparison of potencies cannot be made without knowledge of the relative efflux rates of these substances from each micropipette, in relation to the current applied⁸.

The tripeptide His-(Gly, Lys) thus has inhibitory effects on some neurones in the dorsal horn of the spinal cord and in the medial brainstem, including the reticular formation. Although it was found in the lumbar dorsal columns it has little effect in the dorsal column nuclei, and since the ascending fibres to these nuclei are excitatory it is unlikely to be concerned in transmission in this system. The dorsal columns do, however contain other fibres, such as propriospinal and reticulospinal⁹, and the peptide may be associated with intersegmental fibres or descending fibres terminating in the dorsal horn. The occurrence of the tripeptide His-(Gly, Lys) in the spinal cord and its actions on selected neurones in the dorsal horn suggest that it may have a functional role in relation to neuronal inhibition in the spinal cord.

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Di- and trimethylated congeners of 7-methylguanine in Sindbis virus mRNA

In the past two years it has become clear that most animal messenger RNA—both cellular¹⁻⁶ and viral⁷⁻¹¹—is "capped" by 7-methylguanosine (m⁷G) residues present in inverted nucleotide linkage at 5' termini of the general form m⁷G (5')pppNm. At least for certain mRNAs the capping m⁷G has an important role in mRNA-ribosome binding and thus in initiation of protein synthesis¹²⁻¹⁵. We now report novel forms of capped 5' termini on the main mRNA, '26S' RNA, coded for by the animal alphavirus, Sindbis. These termini contain, in addition to the usual m⁷G, its hypermodified congeners m₂^{2,7}G and m₃^{2,2,7}G. This last compound has been previously described only in small nuclear (sn) RNAs of unknown function^{16,17}, whereas m₂^{2,7}G has not been described previously.

Caps were obtained from methyl-³H-methionine-labelled 26S RNA, using T2-ribonuclease followed by DEAE-cellulose chromatography¹⁸. The recovered oligonucleotides were degraded to ribosides using bacterial alkaline phosphatase and snake venom phosphodiesterase¹; only the extreme 5'-terminal ribosides of such oligonucleotides were labelled, since 26S RNA lacks penultimate Nm residues¹⁸. Preliminary analyses indicated the presence of three methyl-labelled species: m⁷G plus two other ribosides whose properties suggested that they were also 7-methylated derivatives of guanosine, possibly m₂^{2,7}G and m₃^{2,2,7}G (data not shown). Accordingly, we prepared marker compounds by reacting m²G and m₂²G with dimethyl sulphate in conditions known to result in

preferential methylation at the 7 position^{16,19}. Evidence confirming that the products were indeed the expected m₂^{2,7}G and m₃^{2,2,7}G is provided by mass spectra of their free bases (Fig. 1). The most intense peak in the spectrum of both compounds is that of the molecular ion. The assignment of major peaks in Fig. 1a is: *m/e* 179, the molecular ion M; *m/e* 123, 95, 68, arising from the successive loss of cyanamide fragment (CH₃)HNCN, CO and HCN; *m/e* 150, possibly by the loss of CH₃N. The assignment of major peaks in Fig. 1b is: *m/e* 193, the molecular ion M; *m/e* 123, 95, 68, resulting from sequential expulsion of cyanamide fragment (CH₃)₂NCN, CO, and HCN; *m/e* 178, resulting from the loss of one methyl group; and *m/e* 164, possibly arising from expulsion of CH₃N. The loss of the methylamino group resembles that of *N*⁶-methylated and *N*⁶-dimethylated derivatives of adenine. The initial expulsion of a neutral cyanamide fragment with sequential elimination of CO and HCN is highly characteristic of methylated guanines²⁰. Below *m/e* 120, the mass spectra of both m₂^{2,7}Gua and m₃^{2,2,7}Gua are qualitatively similar to that of m⁷Gua (refs 20 and 21). For precise comparison with ³H-labelled samples from 26S RNA we also prepared ¹⁴C-methyl-labelled m₂^{2,7}G and m₃^{2,2,7}G in the same manner.

Figure 2 shows a phosphocellulose elution pattern obtained for the methylated ribosides of the 26S RNA cap, following the procedure used in the original isolation of m₃^{2,2,7}G (ref. 16). The two unknown peaks (I and II in Fig. 2) correspond precisely to the markers m₃^{2,2,7}G and m₂^{2,7}G, respectively. The third major ³H peak III corresponds, as expected, to m⁷G. The radioactivity in the void volume (V) probably represents partial degradation products of the relatively labile 7-methyl-purine ribosides^{16,23,24}.

Table 1 summarises the behaviour of m⁷G, m₂^{2,7}G, and m₃^{2,2,7}G, and their bases, in four other systems. In all cases the ³H from peaks I and II (Fig. 1), or the corresponding bases released by acid hydrolysis, ran with m₃^{2,2,7}G and m₂^{2,7}G, respectively, or their corresponding bases (these latter were the 'unidentified' bases in our initial report¹⁸ on acid hydrolysates of 26S RNA). We conclude that ribosides I and II are indeed m₃^{2,2,7}G and m₂^{2,7}G, respectively.

The m₃^{2,2,7}G residues of snRNAs U-1 and U-2 have been shown to occur as caps on the 5'-terminal sequence m₃^{2,2,7}G (5')pppAmUm . . . (refs 25 and 26), which resembles that of 26S RNA, mG (5')pppApUp . . . (ref. 27 and Dubin *et al.*, unpublished). Conceivably these two forms of RNA, although apparently grossly different in biological and chemical properties, are substrates for the same or related methylases. Another possibility suggested by the presence of similar caps

Fig. 1 Mass spectra of m₂^{2,7}Gua (a) and m₃^{2,2,7}Gua (b). Dried samples of ribosides prepared as described in the text were dissolved in 1 N HCl, heated for 45 min at 100 °C, adjusted to pH 8.5 with concentrated NH₄OH, and lyophilised to dryness. The mass spectral analysis was carried out at 70 eV using a direct inlet system at a temperature of 270 °C.

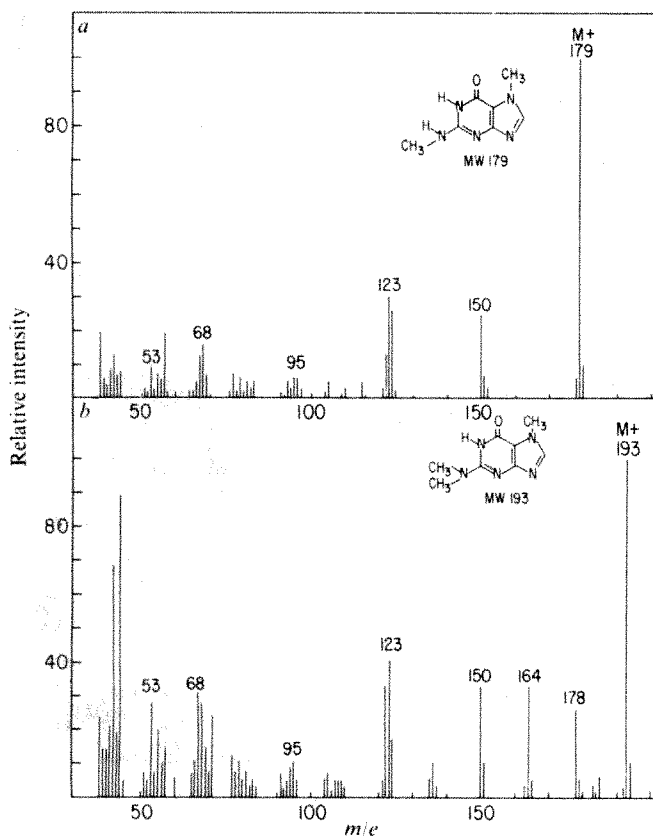


Table 1 Chromatography and electrophoresis of 7-methylated guanines and guanosines

	Mobility or <i>R_f</i> relative to adenine Electrophoresis, pH 3.5	Solvent system		
		A	B	C
m ⁷ G	1.18	1.25	0.15	0.54
m ₂ ^{2,7} G	1.18	2.06	0.45	0.89
m ₃ ^{2,2,7} G	1.18	1.85	0.89	1.33
m ⁷ Gua	0.50	0.85	0.57	—
m ₂ ^{2,7} Gua	0.55	1.93	1.06	—
m ₃ ^{2,2,7} Gua	0.73	1.70	1.12	—

Electrophoresis and chromatography were carried out as described previously^{1,18}. Chromatographic systems were: A, Whatman 3MM paper developed in isopropanol-11.6 N HCl-H₂O (60:17.6:22.4, by volume); B, Whatman 3MM paper developed in *n*-BuOH-H₂O (86:14, by volume in NH₃ atmosphere); C, thin-layer cellulose sheets developed in ethyl acetate-isopropanol-7.5 M NH₄OH-*n*-BuOH (3:2:2:1, by volume). For convenience we have expressed values relative to adenine, which is readily available and which moves well in all systems. *R_f* for adenine in systems A, B and C were about 0.29, 0.25 and 0.40; it ran about 20 cm towards the cathode in 3 h at 3,000 V. Abbreviations follow conventional usage (for example, m⁷Gua, 7-methylguanine; m⁷G, 7-methylguanosine).

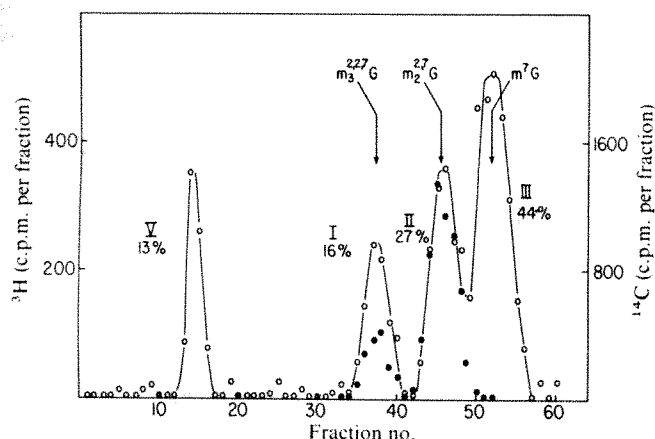


Fig. 2 Phosphocellulose pattern of methylated ribosides from the Sindbis virus 26S RNA cap. The 5'-terminal oligonucleotide of methyl-³H-methionine-labelled Sindbis 26S RNA was prepared from infected chick cells and digested to its constituent ribosides as described previously^{1,18,22}. The digest was chromatographed on a phosphocellulose column using a linear gradient of 0.01–0.3 M ammonium acetate¹⁸. As markers we added ¹⁴C-methyl-labelled $m_3^{2,2,7}$ G and $m_3^{2,2,7}$ G and unlabelled m_7 G. Arrows indicate the midpoints of the 260-nm absorbancy peaks of the markers, and the arabic numeral next to each peak indicates the percentage of the endgroup ³H present in that peak. ○, ³H; ●, ¹⁴C.

on sn and 26S RNAs is that our hypermethylated congeners—or at least $m_3^{2,2,7}$ G—are not in 26S RNA proper but rather occur in snRNA–26S RNA complexes. We believe this is unlikely, since 26S RNA lacks the Nm groups that are abundant in snRNA; our infected cells were labelled in the presence of a level of actinomycin ($4 \mu\text{g ml}^{-1}$, ref. 18) that should substantially suppress cellular RNA synthesis; and recent studies (Dubin *et al.*, unpublished) indicate the presence of 1 mol m_7 G plus congeners per 26S RNA molecule.

There is at present no evidence for a biological role of the extra methyl groups of the 26S RNA capping residues. We are currently attempting to explore one obvious possibility—that they enhance affinity to ribosomes—by comparing poly-some-bound and free 26S RNA.

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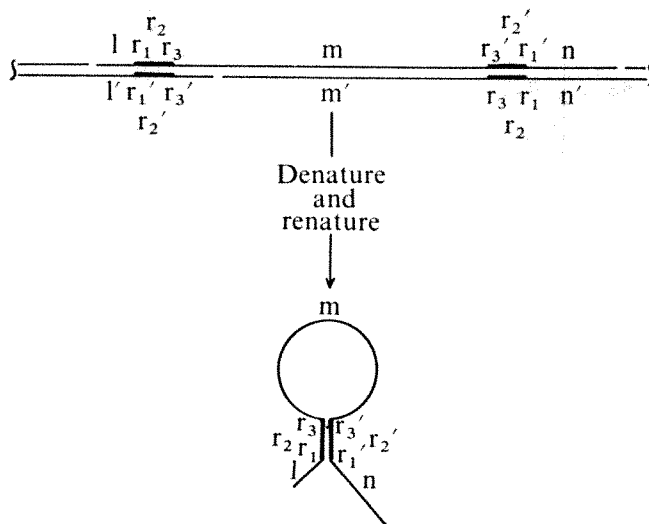
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Distribution of inverted IS-length sequences in the *E. coli* K12 genome

THE genomes of *E. coli* and some of its plasmids contain a class of elements called insertion sequences (IS)^{1–5}. These elements exhibit various properties associated with modulation and alteration of gene expression. IS1 (length 800 nucleotide pairs^{2,3}) apparently plays a role in the amplification of resistance determinants in drug resistance factors^{5,6} and also causes polar insertion mutations^{2,3}. IS2 (length 1,400 nucleotide pairs²) behaves as a controlling element, which can turn gene expression on or off⁷. IS3 (length 1,400 nucleotide pairs³) has been associated with strong polar mutations, and is a constituent of a transposable drug resistance determinant⁸. IS2 and IS3 have also been shown to specify integration loci for the fertility plasmid F^{4,9,10}. Hybridisation data have allowed estimation of the number of IS1 and IS2 elements in the *E. coli* genome¹, but except for the region around *lac* and *purE*, little is known about their natural location in the genome.

While investigating the sequence organisation of three Hfr strains of *E. coli*, we encountered a number of intrastrand hybrid structures which provide information on the distribution of inverted sequences within the *E. coli* K12 genome. Figure 1

Fig. 1 Formation of intrastrand DNA hybrid structures. The upper portion of the figure depicts a slightly nicked DNA duplex. The letters each denote a block of nucleotides; the sequences complementary to each block are indicated by the corresponding primed letter. The sequence $r_1r_2r_3$ is repeated on the DNA molecule in inverted order. Random nicking of DNA duplex (or shear breakage of double-strand or single-strand DNA) produces some strands which have both the sequences $r_1r_2r_3$ and the complementary sequences $r_3'r_2'r_1'$, as indicated for the upper strand. After denaturation and the onset of annealing, the inverted repetitions on such strands can rapidly form intrastrand hybrids, since $r_1r_2r_3$ and $r_3'r_2'r_1'$ are physically linked. Other strands, like the lower one, contain breaks between the inverted sequences, and in such cases, the inverted repetitions will renature slowly since bimolecular processes are required. The intrastrand hybrid structure formed from the upper strand is shown at the bottom. The length of the duplex stem corresponds to the length of the inverted repetition, and the length of the single-strand loop indicates the spacing between inverted repeats.



shows that if a pair of sequences is present as an inverted repetition, denaturation and subsequent reannealing of the DNA may result in an intrastrand hybrid structure. The repeated sequences form a duplex stem whose length corresponds to the length of the repeated sequence. The DNA separating the inverted sequences appears as a single strand loop whose length corresponds to the distance separating the inverted repetitions. Directly repeated sequences will not form intrastrand duplexes. Figures 2 and 3 are electron micrographs of two different intrastrand hybrid structures obtained from the DNA of strain P804. Similar structures were seen with DNA extracted from the strains P4X and P3. In both figures, the length of the duplex stem is approximately 1,300 nucleotide pairs (1.3 kilobases, or kb).

Three size classes of single-strand loops were detected (Table 1). There were only two representatives of the largest size class. Intrastrand hybrid structures with larger loops were less abundant than structures with smaller loops. Since renaturation times were long, the difference in abundance of these intrastrand structures is unlikely to be the result of kinetic effects. Because DNA strand lengths for a typical preparation were 120 ± 60 kb, inverted repetitions separated by more than 180 kb could form loop structures only rarely. Inverted repetitions located far from each other are more likely to be separated by strand breaks between the inverted sequences than are closely linked inverted repetitions, and therefore larger loop sizes will appear less frequently than smaller ones, even though the more widely spaced inverted sequences may appear in the genome at the same frequency as the less widely spaced ones. This relative enrichment for smaller loop sizes is reflected in Table 1.

These experiments do not identify the DNA contained in the duplex stems; however, the stem sizes are approximately

Fig. 2 Intrastrand hybrid structure from *E. coli* K12 strain P804. Folded chromosomes were isolated by using modifications of the methods of Worcel and Burgi^{10,15}. Bacterial DNA was denatured by incubation in 0.33 M NaOH for 5 min at 37 °C, and then for 10 min at room temperature. The sample was neutralised and dialysed at room temperature for 2–3 h against 70% formamide, 0.25 M NaCl, 0.10 M Tris, 0.01 M EDTA, pH 8.5, for the reannealing step. The concentration of the bacterial DNA was approximately $1.5 \mu\text{g ml}^{-1}$. Renaturation was terminated by dialysis at 4 °C against 50% formamide, 0.10 M Tris, 0.01 M EDTA pH 8.5. DNA was mounted from 55% formamide by the basic protein film technique as previously described¹⁶. The arrow in the micrograph points to the duplex stem, whose length is 1.2 kb. The single-strand loop has a length of 29 kb. The scale bar indicates the distance corresponding to 1 kb (1,000 nucleotides of single-strand DNA or 1,000 nucleotide pairs of double-strand DNA). Circular reference molecules are from ϕX174 .

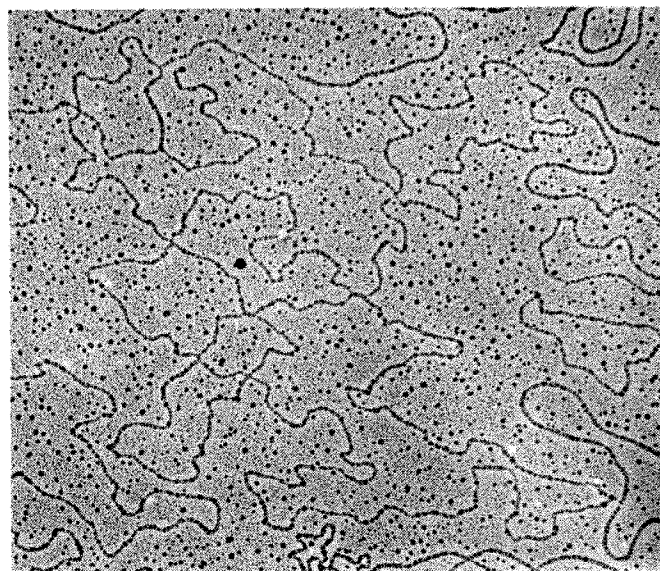
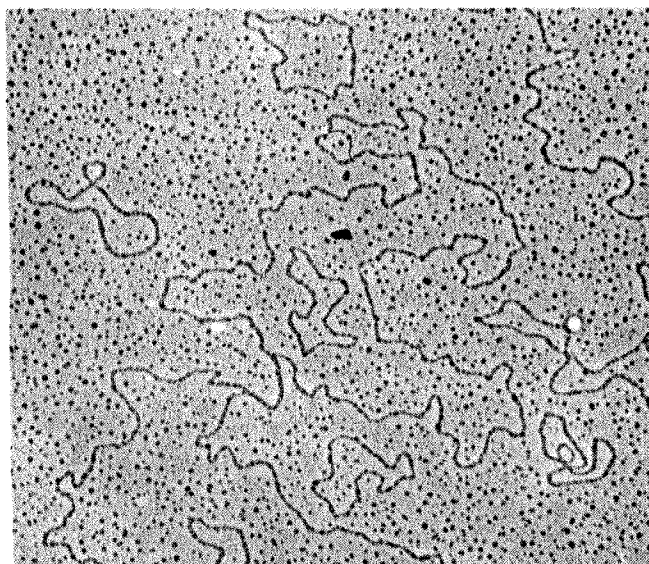


Fig. 3 Intrastrand hybrid structure from *E. coli* K12 strain P804. The stem (indicated by the arrow) has a length of 1.3 kb, and the loop has a length of 69 kb.

the same size as both IS2 and IS3. The duplex stem length associated with the 69-kb loops is 1.3 ± 0.1 kb, while the stem length for the 29-kb loop structure is 1.2 ± 0.2 kb. Histograms of stem lengths are shown in Fig. 4. The histogram for hybrid regions from the 29-kb structures (Fig. 4a) displays a shoulder corresponding to shortened stem lengths. Such shortening could result from partial melting of a partially mismatched structure, or it could indicate that the stems of the 29-kb loops are shorter and therefore different from the stems of the 69-kb loops.

Since the 0.8-kb IS1 element is known to be present in the *E. coli* genome¹, there is the possibility that intrastrand hybrid structures involving IS1 might form. Three intrastrand structures with duplex stems shorter than 1 kb (and distinct from the structures with 29-kb loops) were detected, but each displayed a different loop size. We would have detected any structure classes having hybrid portions in the 0.75–1.00-kb size range had they been present at frequencies comparable with those of the other structures. As Fig. 4a shows, a few examples of the 29-kb structure with duplex regions in this size range were seen, which confirms that our specimen scanning procedures were sensitive to this size range. We conclude that

Table 1 Distances separating 1.3-kb inverted repetitions in three strains of *E. coli* K12

Strain	Loop sizes (kb)		
	I	II	III
P3	29.4 ± 2.6 (11)	67.4 ± 3.8 (9)	184.7* (1)
P4X	29.5 ± 1.9 (6)	73.1 ± 4.1 (4)	—
P804	29.3 ± 1.4 (10)	69.6 ± 2.6 (5)	173.1 (1)
Average	29.4 ± 2.0	69.3 ± 4.1	

Intrastrand hybrid structures were detected by scanning denatured and reannealed preparations of *E. coli* DNA in the electron microscope. Sizes were calculated by using ϕX174 DNA mounted on the same grid as a size reference (taken to be 5.25 kb). The numbers of structures measured are given in parentheses.

*This structure contained two hybrid regions whose sizes and relative separation suggested the participation of sequences from the integrated F plasmid.

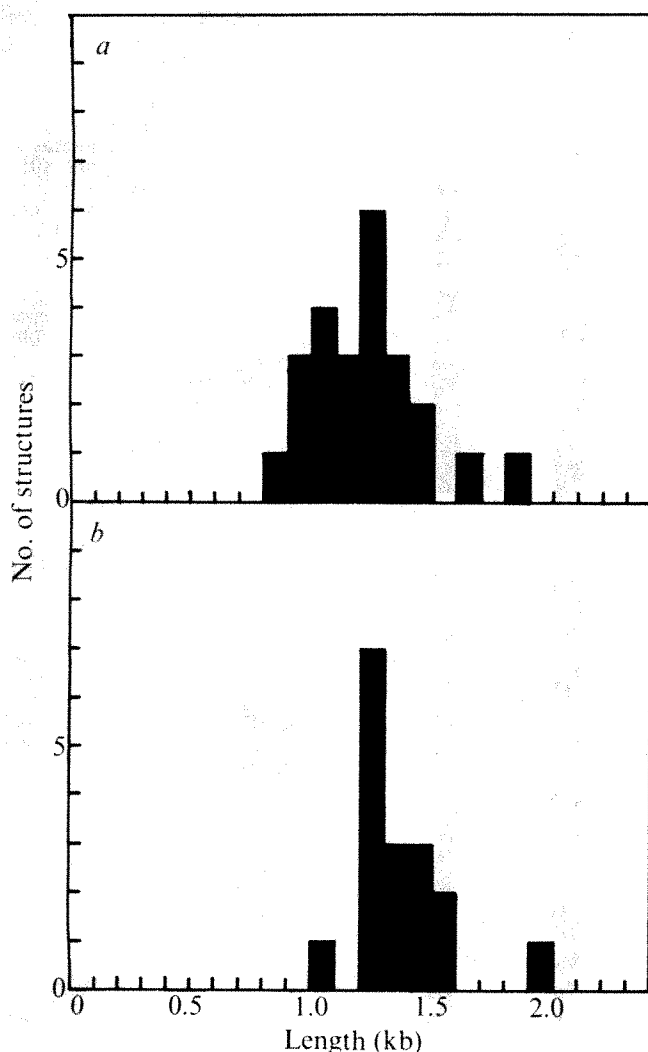


Fig. 4 Histograms of lengths of duplex hybrid regions for the major loop size classes: *a*, hybrid lengths associated with the 29.4-kb loop; *b*, hybrid lengths associated with the 69.3-kb loop.

there are probably few IS1 length inverted repetitions relatively close together in the strains which we have examined; however, we cannot rule out the possibility that our reannealing or mounting conditions are somehow unfavourable for formation or retention of such structures. It is important to note that inverted repetitions longer than 1.3 kb would have been detected if they were sufficiently close. No such structures were observed.

In addition to the inverted repeat structures described, more complicated examples containing two hybrid regions were seen, but these are attributable to participation of IS2 and IS3 elements present on the integrated F factor in these Hfr strains. It should be emphasised that the organisation of the integrated F in these three strains is known¹⁰ (our unpublished results), as are the structures of the added F and F-prime heteroduplex reference molecules which were present during these experiments. The structures which appear in Figs 2 and 3 are not characteristic of the F or F-prime reference DNA which was also present. The main size classes seen in strain P3 are found also in P4X and P804, even though the site of the integrated F factor in P3 is some 240 kb removed from the F integration site in P4X and P804. This provides further evidence that these inverted repeat structures are not dependent on the Hfr character of these strains.

The data presented here provide information on the distribution of IS2 and IS3 size inverted repetitions in the *E. coli*

K12 genome. Previous heteroduplex mapping of the F-prime F13, which carries the *lac-purE* region from *E. coli*, identified three IS3 elements and one IS2 in the region⁹. If these elements are normal constituents of *E. coli* K12, then the 69-kb loop is predictable from separation distances and orientations of IS3 elements in the *lac-purE* region. The location of the DNA contained in the smallest loop is unknown.

We can conclude that elements similar in structure and size to the *tet*^R transposon⁸ and the *kan-2* elements¹¹, which have flanking inverted repetitions of 1.4 kb, are absent in the strains which we have examined. The loop sizes which are seen in intrastrand hybrid structures containing these elements are in the 5–10-kb size range, and we would surely have detected them. Elements structurally similar to the TEM β -lactamase transposon TnA¹² would not have been detected in these experiments, since the short stems characteristic of their intrastrand hybrid structures would have looked like overlaps.

The Hfr strains P4X and P3 are closely related¹³, and they exhibit similar loop sizes. P804 is less closely related to P3 than is P4X¹³, yet the size classes correspond to those of P3 and P4X. This again suggests that these inverted elements are relatively stable components of the *E. coli* genome, as has been suggested for IS3¹⁴.

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Cleavage map of colicin E1 plasmid

COLICIN E1 plasmid (colE1) is a closed circular DNA molecule with a molecular weight of 4.2×10^6 (ref. 1). ColE1 DNA has extensively been used as a molecular vehicle for cloning and amplification of DNA in genetic engineering^{2,3}. In order to expand such investigations, it is useful to make a cleavage map ordering colE1 DNA pieces produced by bacterial restriction endonucleases. *Escherichia coli* RI restriction endonuclease (R·EcoRI) has been shown to cleave colE1 DNA at a single unique site^{4–6}. We have now constructed a physical map with the EcoRI site as a reference point using two restriction endonucleases from *Haemophilus aegyptius* (R·HaeII and R·HaeIII). The map of the colE1 moiety involved in a hybrid plasmid pML21 (ref. 7) carrying about a half of the colE1 genome was also determined. Restriction endonucleases and fragments pro-

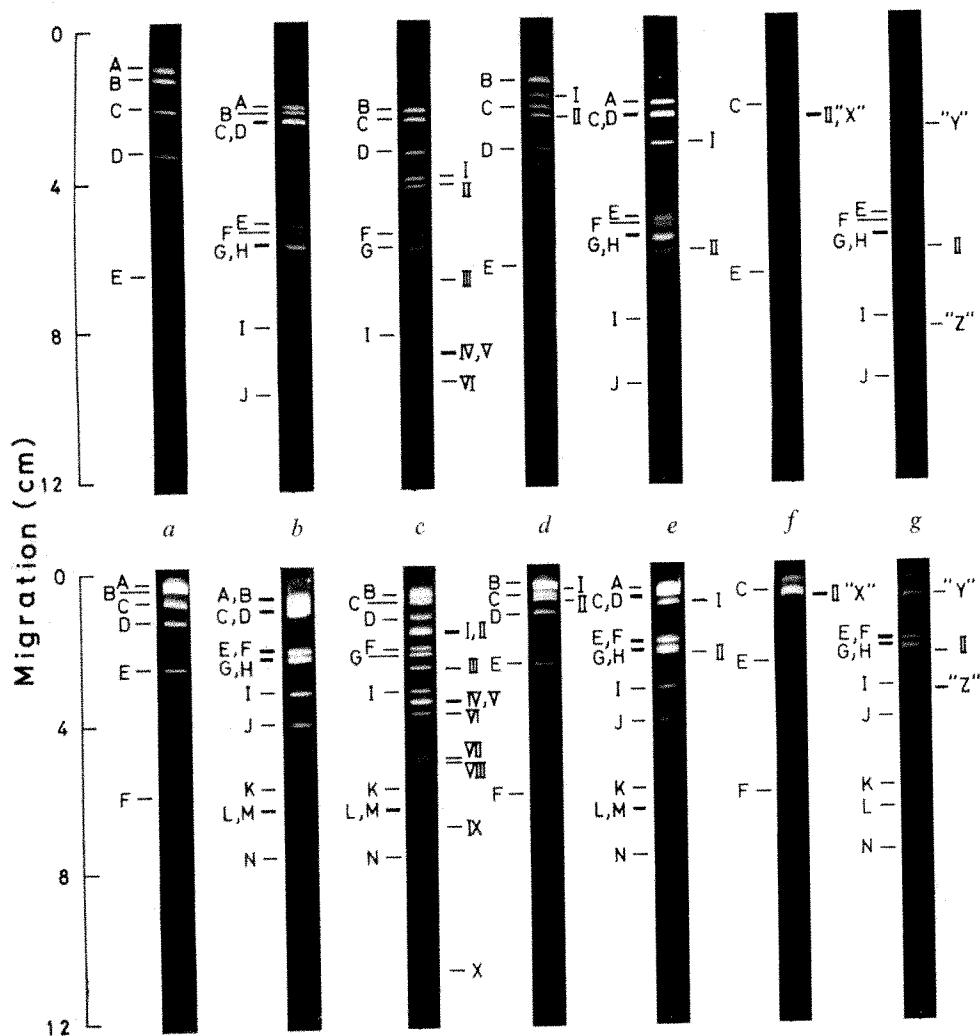


Fig. 1 Gel electrophoretic patterns of digests of colE1 DNA and the mini-colE1 segment of pML21 with restriction endonucleases. ColE1 DNA or the mini-colE1 segment of pML21 was digested with restriction endonucleases, electrophoresed on 5% and 10% polyacrylamide gels (0.6 cm × 12 cm), and the gels were stained with ethidium bromide (0.5 µg ml⁻¹). DNA bands were visualised in long-wave ultraviolet light and photographed using Kodak No. 23A red filter and Polaroid land pack film type 105. Photographs from 5% gels were shown above and those from 10% gels below. *a-e*, Digests of colE1 DNA; *f* and *g*, digests of the mini-colE1 segment of pML21. Restriction endonucleases used were: (*a*) R·HaeII, (*b*) R·HaeIII, (*c*) R·HaeII + R·HaeIII, (*d*) R·HaeII + R·EcoRI, (*e*) R·HaeIII + R·EcoRI, (*f*) R·HaeII, and (*g*) R·HaeIII. Capitals on the left side of *a*, *d* and *f* are HaeII fragments and those on *b*, *c*, *e* and *g* are HaeIII fragments, except "D" on *c*, that is the HaeII fragment. Roman numerals on the right side show the colE1 DNA fragments created by cleavages with two different enzymes. "X", "Y" and "Z" on *f* and *g* were the fragments found only in the digests of the mini-colE1 segment of pML21. Preparation of colE1 DNA, isolation of R·HaeII, R·HaeIII and R·EcoRI, digestion of DNA and method of polyacrylamide gel electrophoresis have been described⁹⁻¹². The mini-colE1 segment of pML21 was isolated from the EcoRI digest of pML21 DNA by Agarose gel electrophoresis¹².

duced by the cleavages were abbreviated as those by Smith and Nathans⁸. The size of each fragment was expressed as a percentage of the length of the colE1 DNA molecule.

ColE1 and pML21 DNAs were isolated respectively from *E. coli* P678-54 (colE1) and *E. coli* W3350thy-(pML21), as described earlier^{7,9}. R·HaeII and R·HaeIII were prepared from *H. aegyptius* by the method of Roberts *et al.* and R·EcoRI from *E. coli* RY13 by the method of Greene *et al.*¹². Restriction fragments were resolved by electrophoresis on either 5% or 10% polyacrylamide gel¹⁰ or on 1.2% Agarose gel¹², and visualised by staining with ethidium bromide. For isolation of fragments from gels, band regions visualised by a brief staining were homogenised in a few ml of 10 mM Tris-HCl (pH 7.6) and incubated for 15 h at 37 °C. The extract was concentrated and purified by passing through a Sephadex G-100 column (0.4 cm × 15 cm). The size of HaeII and HaeIII fragments was estimated from the distribution of ³²P in each fragment generated from uniformly labelled ³²P-DNA. The size of other fragments was estimated from the mobility relative to the HaeII and HaeIII fragments.

When colE1 DNA was subjected to complete digestion with R·HaeII, six specific fragments (HaeII-A to -F) were visualised after polyacrylamide gel electrophoresis and subsequent staining of the gels with ethidium bromide (Fig. 1*a*). On the basis of the relative yield of each fragment from uniformly labelled ³²P-DNA, their molecular weights were estimated to be 36.9%, 27.5%, 18.0%, 11.0%, 5.4% and 1.2%, respectively. The order of these fragments was determined from analysis of partial digests followed by redigestion of individual intermediate fragments. A representative electropherogram of partial digests

is presented in Fig. 2, in which several discrete fragments can be seen in addition to the limit digestion products. The approximate size of each band is given by the side of the gel column. DNA banding between HaeII-A and -D was completely digested with R·HaeII and the products were analysed. As a result, it was found that 6 out of 10 bands tested were intermediates of digestion (i1 to i6) and the others were HaeII fragments A, B, C and D. Each intermediate gave limit products as follows: i1 → B D, i2 → C D F, i3 → C E F, i4 → C E, i5 → C F, i6 → D F. By arranging these limit products in overlapping positions, the physical order of the HaeII fragments was deduced to be A E C F D B, with A and B being contiguous in the circular molecule. This order was further confirmed by analysis of the EcoRI site and the constituent HaeII fragments of pML21, as described below.

As one specific break is valuable as a reference point in the circular molecule, the location of the EcoRI cleavage site in the HaeII fragments was determined by comparison between the HaeII digests of the circular colE1 DNA molecule and the EcoRI-treated linear colE1 DNA molecule (EcoRI-A). The only difference in the two digests was the absence of HaeII-A among the EcoRI·HaeII products, and the appearance of two new fragments: one between HaeII-B and -C and the other between HaeII-C and -D (I and II in Fig. 1*d*). The EcoRI site was therefore within HaeII-A. This was confirmed by direct cleavage of HaeII-A with R·EcoRI (data not shown), the two products I and II being identified as EcoRI-A·HaeII-A1 and EcoRI-A·HaeII-A2, respectively. Their sizes were estimated to be 21.4% and 16.4%, respectively. Which end of HaeII-A is closer to the EcoRI site was determined with pML21 as follows.

Table 1 Digestion of purified *Hae* fragments with the second *Hae* enzyme

Original fragment	Products of the second digestion
<i>Hae</i> II-A	<i>HH</i> -II, <i>HH</i> -IV [†] , <i>Hae</i> III-B, <i>Hae</i> III-F
<i>Hae</i> II-B	<i>HH</i> -I, <i>HH</i> -V [†] , <i>Hae</i> III-C, <i>Hae</i> III-M*
<i>Hae</i> II-C	<i>HH</i> -III, <i>HH</i> -VII, <i>Hae</i> III-G, <i>Hae</i> III-I, <i>Hae</i> III-K, <i>Hae</i> III-L*
<i>Hae</i> II-E	<i>HH</i> -VI, <i>HH</i> -VIII, <i>Hae</i> III-N
<i>Hae</i> II-F	<i>HH</i> -IX [§] , <i>HH</i> -X [§]
<i>Hae</i> III-A	<i>HH</i> -I, <i>HH</i> -II
<i>Hae</i> III-D [†]	<i>HH</i> -V [†] , <i>HH</i> -X, <i>Hae</i> II-D
<i>Hae</i> III-E	<i>HH</i> -III, <i>HH</i> -VIII
<i>Hae</i> III-H [†]	<i>HH</i> -IV [†] , <i>HH</i> -VI
<i>Hae</i> III-J	<i>HH</i> -VII, <i>HH</i> -IX

Each *Hae*II and *Hae*III fragment shown in the first column was purified by polyacrylamide gel electrophoresis and completely digested with the second *Hae* enzyme. Limit products determined by gel electrophoresis are shown in the second column. In parallel experiments, *Hae*II-D, *Hae*III-B, *Hae*III-C, *Hae*III-F, *Hae*III-G and *Hae*III-I were confirmed to carry no *Hae* cleavage site inside.

*The sizes of *Hae*III-L and *Hae*III-M were about equal, one derived from *Hae*II-C was named *Hae*III-L.

†Digestion of *Hae*III-D and *Hae*III-H with R-*Hae*II was done in the presence of *Hae*III-C and *Hae*III-G, respectively.

‡*HH*-IV and *HH*-V were indistinguishable by gel electrophoresis; one derived from *Hae*II-A was named *HH*-IV. This *HH* fragment should be identical to the one produced from *Hae*III-H, because analysis of overlapping positions at the other end indicates that *Hae*II-A partly overlaps with *Hae*III-H but not with *Hae*III-D. Similarly, the other overlapping fragment produced from *Hae*II-B and *Hae*III-D was named *HH*-V.

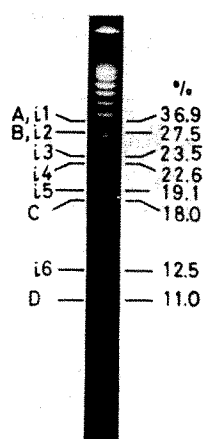
§Although not directly demonstrated, *HH*-IX and *HH*-X are evidently derived from *Hae*II-F because the total length of these two fragments is comparable to that of *Hae*II-F and there is no other candidate yielding these fragments.

As previously described^{7,13}, this plasmid replicates as the *colE1* replicon, and yields two fragments when digested with R-*Eco*RI: the longer one contains kanamycin resistance marker but no *colE1* DNA, and the shorter one, defined as the mini-*colE1* segment, continuously carries 49% of the *colE1* genome and a small amount of non-*colE1* DNA sequence (about 5% length of *colE1* DNA). One end of the latter fragment is precisely homologous to one end of the linear *colE1* DNA generated by the cleavage with R-*Eco*RI. Therefore, pML21 should carry one of the two *Eco*RI-A-*Hae*II-A fragments of *colE1*. When the mini-*colE1* segment of pML21 was digested with R-*Hae*II, *Eco*RI-A-*Hae*II-A2 in addition to *Hae*II-C, -E, -F and a new fragment "X" were produced, but not *Eco*RI-A-*Hae*II-A1, *Hae*II-B and -D (compare Fig. 1d and f). The "X" fragment was interpreted to carry a part of *Hae*II-D and the non-*colE1* DNA in the mini-*colE1* segment of pML21 from its electrophoretic

mobility. It was thus concluded that *Eco*RI-A-*Hae*II-A2 was next to *Hae*II-E, confirming that the fragment linked to *Hae*II-E was *Hae*II-A but never *Hae*II-B.

Since use of different restriction endonuclease for fragment analyses at overlapping positions strengthens fidelity of the physical map, digestion of *colE1* DNA with another enzyme R-*Hae*III was performed. R-*Hae*III cleaved *colE1* DNA into 14 pieces (*Hae*III-A to -N in Fig. 1b), in which C and D, G and H, and L and M were respectively inseparable in the conditions used. The molecular weights for *Hae*III fragments (A to N) estimated as in the case of *Hae*II fragments were 17.3%, 16.6%, 14.3%, 14.3%, 6.9%, 6.7%, 6.5%, 6.5%, 4.0%, 2.7%, 1.2%, 1.1%, 1.1%, and 0.8%, respectively. Combined digestion of *colE1* DNA with R-*Hae*II and R-*Hae*III gave 20 pieces as expected from additivity of the number of cleavage sites (Fig. 1c). From comparison between the gel electropherograms of the digests of *colE1* DNA with both R-*Hae*II and R-*Hae*III and with either one, it is evident that 10 out of 20 fragments correspond to *Hae*II-D, *Hae*III-B, -C (or -D), -F, -G (or -H), -I, -K, -L, -M, and -N. This was confirmed by direct digestion of each fragment with the second *Hae* enzyme (see below). As the sizes of *Hae*III-C and -D, and *Hae*III-G and -H were about equal, the fragments carrying no *Hae*II cleavage site were named *Hae*III-C and -G, respectively. It was thus concluded that *Hae*II sites were present in *Hae*III-A, -D, -E, -H and -J, and that all *Hae*II fragments except *Hae*II-D contained *Hae*III substrate sites. The remaining 10 fragments, *Hae*II-*Hae*III-I to -X (designated as *HH*-I to -X), were produced by cleavages with *Hae*II at one end and with *Hae*III at the other end. Their sizes estimated from their electrophoretic mobilities were 9.1%, 8.6%, 5.3%, 3.3%, 3.3%, 2.9%, 1.7%, 1.6%, 0.9% and 0.4%, respectively. To analyse which *Hae*III fragments were contained in a particular *Hae*II fragment, and vice versa, and from which *Hae*II and *Hae*III fragments a particular *HH* fragment was derived, each *Hae*II and *Hae*III fragment except small fragments (that is *Hae*II-F and *Hae*III-K to -N) were purified and digested with the second enzyme, and the fragments produced were determined. The results are summarised in Table 1, from which we can deduce origins of all *HH* fragments and, therefore, relate the two sets of fragments as follows:

Fig. 2 A gel electrophoretic pattern of partial digests of *colE1* DNA with R-*Hae*II. *ColE1* DNA was partially digested with R-*Hae*II by reducing the amount of enzyme, and subjected to electrophoresis on a 5% polyacrylamide gel. Since no partial digestion product with electrophoretic mobility faster than that of *Hae*II-D was found in preliminary experiments, electrophoresis was done at about twice the current of that in Fig. 1 for obtaining good separation. Capitals indicate the band positions of *Hae*II fragments, and i1 to i6 are intermediate fragments of digestion. The approximate size of each band is given on the right-hand side. Since *Hae*II-A and i1, and *Hae*II-B and i2 were respectively too close to isolate, redigestion was performed on the mixture.



<i>Hae</i> II	A	B	D	F	C	E	A
<i>HH</i>	II	I	V	X IX VII	III VIII	VI IV	
<i>Hae</i> III	A	(C, M)	D	J	(G, I, K, L)	E	N H (B, F)

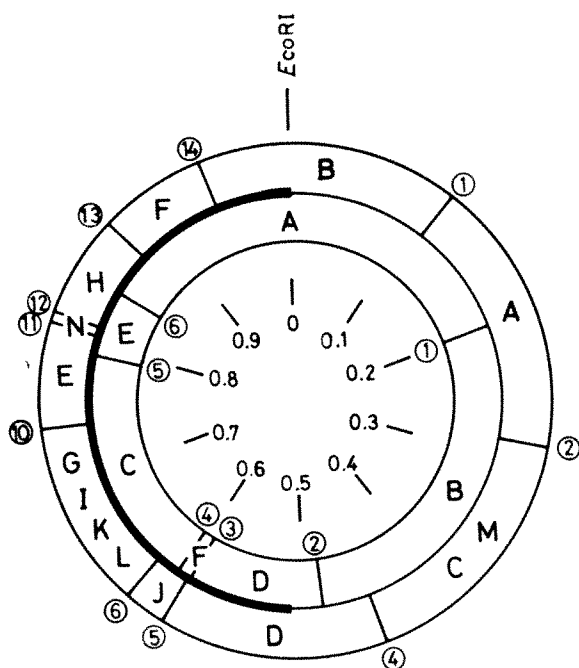


Fig. 3 Physical map of the colE1 genome constructed by the cleavages with R·EcoRI, R·HaeII and R·HaeIII. The inner and outer rings show HaeII and HaeIII fragments, respectively. The EcoRI cleavage site was designated the zero point, and each cleavage site was numbered in the clockwise direction (circled numbers). The map distance from the zero point is shown as the fraction of the length of colE1 DNA in the same direction. Heavy line indicates the region carried by pML21.

To determine the positions of HaeIII-B and -F in HaeII-A, the location of the EcoRI site in HaeIII fragments and the constituent HaeIII fragments of pML21 were analysed. The electropherogram of combined digests of colE1 DNA with R·EcoRI and R·HaeIII indicates that HaeIII-B was split with R·EcoRI into two fragments, EcoRI-A·HaeIII-B1 (11.4%) and EcoRI-A·HaeIII-B2 (5.9%) (I and II in Fig. 1e). Digestion of the mini-colE1 segment of pML21 with R·HaeIII yielded EcoRI-A·HaeIII-B2, HaeIII-E, -F, -G, -H, -I, -J, -K, -L, -N and two new fragments "Y" and "Z" (Fig. 1g). Thus it was concluded that EcoRI-A·HaeIII-B2 and HaeIII-F were within EcoRI-A·HaeII-A2. The appearance of the two new fragments indicates that non-colE1 DNA in the mini-colE1 segment of pML21 contains one HaeIII site.

We can usefully summarise all the foregoing data in a single cleavage map of the colE1 genome, incorporating the various cleavage sites and molecular weight estimates of the fragments (Fig. 3). The EcoRI site was designated the zero point and measurement of the map distance as the fraction of the length of colE1 DNA was made in the direction A B D F C E of HaeII fragments from this point. According to this expression, pML21 carries the region 0.51 to 1.0. Earlier work indicates that replication initiates at 18% distant from the EcoRI site and proceeds towards the distal EcoRI site⁴⁻⁶. The replication origin of colE1 is present in pML21 (ref. 7). Therefore, the origin and direction of DNA replication can be deduced to be 0.82 and anticlockwise on the map in Fig. 3.

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Corrigendum

In the article "Prehistoric exploration at Hadar, Ethiopia" by G. Corvinus (*Nature*, **261**, 571; 1976) no acknowledgement was given to the institution which funded the work. The author wishes to thank the Centre National de Recherche Scientifique for a grant in 1974 and for a fellowship in 1975.

Errata

In the article "Reactions involving singlet oxygen anion" by W. H. Koppenol (*Nature*, **262**, 420; 1976) for 'effects', page 420, paragraph 1, line 6, read 'involvement'. Also, page 421, line 29, insert 'quantitatively' between 'reacts' and 'with'.

In the article "New cranium of *Homo erectus* from Lake Ndutu, Tanzania" by R. J. Clarke (*Nature*, **262**, 485; 1976) the following corrections should be made. In the first paragraph on page 485 humanoid should read hominid. The semicolon in the third line of the second paragraph on page 486 should be replaced by a comma and the last word in the same line should be 'lacking'. The last sentence in the same paragraph should read 'In addition, there are the isolated right tympanic plate and both isolated petrous temporals'. There is no scale on Fig. 1: the distance from a to O is 12 cm.

In the article "RNA polymerase specificity and the control of growth" by A. Travers (*Nature*, **263**, 641; 1976) the following corrections should be made on page 643: Paragraph 3, the sentence beginning in line 16 should read '... addition of T7 DNA results in a reduction of T2 RNA synthesis and a stimulation ...'; and the sentence beginning in line 28 should read '... the relative competition of T2 RNA and rRNA synthesis is virtually independent of the nature of the competing template.'; and in paragraph 6, the second sentence should read 'In two instances where stable RNA synthesis is shut off *in vivo* ppGpp accumulation and T4 infection ...'.

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matters arising

Asymmetric degradation of DL-leucine with longitudinally polarised electrons

BONNER *et al.* reported that when DL-leucine was irradiated with natural, antiparallel, polarised electrons of 120 keV energy, D-leucine was decomposed to a greater extent than L-leucine: with unnatural, parallel, polarised electron irradiation, however, L-leucine was degraded to a greater extent¹.

The mechanism of this phenomenon is not known. The authors were unable to decide whether the asymmetric degradations was caused by the longitudinally polarised electrons themselves, or by their bremsstrahlung. I wish to point out that the degradations measured could not have been caused by the bremsstrahlung of the bombarding electrons.

The fact that $\Delta E = 29$ eV for three cases is remarkable and shows that the law of exponential decomposition is applicable in the analysis. The value of ΔE coincides with the same values in the case of bremsstrahlung irradiation³. The ΔE value is very reasonable, showing that there is no need to assume any chain-type reaction mechanism.

The values of relative difference in the decomposition rates ($2Z$) show a broader spread. This, and the large value of ΔE in experiment (2), can be understood by taking into account the remarks of Bonner *et al.*, that it was difficult to control many of the experimental parameters of the polarised electron source. Even so, I feel that the value $2Z \approx 10\%$ can be accepted. (The upper limit for the same quantity in the case of DL-alanine was $2Z \approx 0.1\%$ (ref. 4).)

The energy of the incoming electrons

$2Z \approx 10\%$. A mechanism based on the direct interaction of polarised electrons with the asymmetric molecules, which was first outlined in ref. 6 and needs to be worked out in detail, may provide an approach to the understanding of asymmetric degradation.

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Table 1 Analysis of the asymmetric degradation of DL-leucine

Experiment	Irradiation nA h	Sample decomposition	Energy/decomposed molecule (eV)	$\frac{\epsilon_D - \epsilon_L}{\epsilon_0}$	Weighted average
(1)	331	52.9	29.3	0.189 ± 0.039	0.151 ± 0.024
(2)	498	50.9	55	0.129 ± 0.030	
(3)	795	73.9	29	0.085 ± 0.015	0.081 ± 0.012
(4)	860	75.6	29	0.074 ± 0.020	

Analysing the data given in Table 1, of ref. 1, I calculated the average energy spent by the electrons to decompose a single leucine molecule (ΔE) and the relative difference of the decomposition rates for L- and D-leucine ($2Z = (\epsilon_L - \epsilon_D)/\epsilon_0$). For this purpose the following simple equation was used (which assumes an exponential decomposition of the molecules under constant irradiation²)

$$\frac{N_L - N_D}{N_L + N_D} = PZ \ln \frac{1}{1-D} \quad (1)$$

where the left side is the relative difference in the number of remaining L and D molecules, that is, the enantiomeric selectivity ($\%L - \%D$) defined in ref. 1; P is the polarisation of the incoming electron beam (percentage excess in Table 1 of ref. 1); and D is the sample decomposition (percentage divided by 100). The thickness of the irradiated DL-leucine samples (~ 20 mg cm⁻²) was nearly equal to the range of the bombarding electrons.

The data are presented in Table 1.

is lost in the sample by ionisation and radiation; the latter is the bremsstrahlung.

The fraction of the incident energy which is converted into bremsstrahlung in a thick target is approximately⁵

$$I/E = 7ZE \times 10^{-4} \quad (2)$$

where Z is the atomic number of the bombarded material (~ 7) and E is the energy of electrons in MeV.

Even if the whole energy of the bremsstrahlung had been absorbed in the sample, less than 0.1% of the degraded molecules would have been decomposed by bremsstrahlung. If the low degree of polarisation of the low energy bremsstrahlung ($P \approx 10^{-3}$) and the very low upper limit for Z ($< 3 \times 10^{-3}$ according to the measurements in ref. 2) were also taken into account, the upper limit of the enantiomeric selectivity would be $\sim 10^{-6}\%$. It is not possible therefore to understand the great difference found by Bonner *et al.* if the asymmetric degradation is caused by bremsstrahlung. There is no known mechanism to explain the effect of

BONNER ET AL. REPLY—Keszthelyi calculates that the average energy spent by the electrons to decompose a single leucine molecule is about 29 eV, that less than 0.1% of the degradations were due to bremsstrahlung, and that therefore ionisation must have engendered > 99.9% of the degradation as well as (in some unspecified manner) the observed asymmetric effect. We wish to comment on Keszthelyi's interpretation¹ of our results.

Using the sample weight, percentage degradation and total coulombs used in our experiments², one readily calculates that more than 3,000 molecules of DL-leucine were decomposed for each impinging primary electron. If each leucine decomposition requires an average 29 eV (ref. 1), each primary 120-keV electron thus loses an average total of about 87 keV in decomposing the leucine substrate. The remaining 32 keV presumably is lost in decomposing the collodian binder and in the rather inefficient geometry of the leucine target. There are thus some 3,000 primary processes initiated by each incident chiral electron. If primary ionisation is the predominant chiral process, the chiral efficiency of each such event must on average approach 5% to explain the asymmetric effect observed. If, as is possible, the incident electron loses its chirality after fewer collisions, the chiral efficiency must be still greater. What mechanisms might permit this?

Keszthelyi is correct that < 0.1% of the chiral primary electron energy is available as bremsstrahlung, which would

be fully circularly polarised only at the low intensity³, high energy end of its spectrum⁴. Thus it would seem unlikely that circularly polarised bremsstrahlung alone could explain our asymmetric effect² (ignoring such unknown amplification mechanisms as might involve the crystal lattice). There is, however, an additional source of chiral electromagnetic radiation potentially available for asymmetric photolytic decomposition—circularly polarised phosphorescence or fluorescence radiation emitted by the chiral substrate after excitation by the chiral primary electrons⁵. Another alternative amplification mechanism might involve chiral secondary electrons of about 29 eV, liberated through ionisation of the chiral substrate molecules by chiral primary electrons. Such chiral secondary electrons should be quite abundant, that is, about 3,000 per initial chiral primary electron. Experiments which we hope may shed light on these questions are in progress.

Our work was supported in part by the Office of Naval Research, NASA and ERDA. We are grateful to Professor H. P. Noyes (Stanford Linear Accelerator Centre) for his contributions to our reply.

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Fractures and movement in the Adelaide rift zone

FOLD patterns in the Adelaide geosyncline have been interpreted by Coward¹ as evidence of left-lateral shear between two Australian subplates, although the crustal shears have not been defined in detail. It has been implied further that left-lateral movement still exists in this region^{1,2}. Apparently, studies of earthquakes^{3,4} have not been taken into account in the analysis of the tectonics of South Australia, since these indicate that right-lateral shear occurs at present, and may have done so in the past.

The seismicity data quoted¹ contain few events⁵, and only show that seismic activity exists in the region of the rift zone. More recent data suggest that well-defined trends in activity occur², which may outline the main fracture zones in the crust (Fig. 1). From focal mechanisms⁴ and trends in energy release⁶, we propose the tectonic model shown in Fig. 2. This is modified from Stewart and Mount⁴, and may be further altered for the eastern and southern extensions of the rift zone. Present faulting varies from strike slip near Adelaide to dip slip in the area of the Flinders Ranges⁴ (Fig.

2), and is more complex than the simple shear model used to interpret folding¹. The lateral extent of the seismicity trends (Fig. 1) and focal depths³ suggest that the shear zones in Fig. 2 represent fractures extending to the lower crust, and possibly into the upper mantle. The proposed shear zone at the northern end of the rift zone coincides with a region of high conductivity in the deep crust and upper mantle⁷ (Fig. 2). Since such shear zones may be active over extended periods of geological time⁸, the boundaries defined in Fig. 2 may represent the major lines of weakness in the crust throughout the life of the geosyncline (that is, since the late Proterozoic). The shear zones in Fig. 2 are approximately parallel to the sinistral shear couple proposed by Coward¹.

of the Adelaide rift zone (Fig. 2) may be compatible with the north-south compressive stress observed in central Australia⁹.

Widespread diapirism in the northern section of the rift zone (Fig. 2) seems to have occurred in conditions of regional tension (T. J. Mount, personal communication). On the model of Fig. 2, this implies that right-lateral movement occurred between the subplates at the time of diapirism. The intrusion of Proterozoic carbonate material into the overlying cover rocks may have continued from the Upper Proterozoic at least into the Cambrian¹⁰. Thus although left-lateral movement may have occurred at various times in the past, this may not always have been the case, and is probably not so at present. Rather, the movement may be

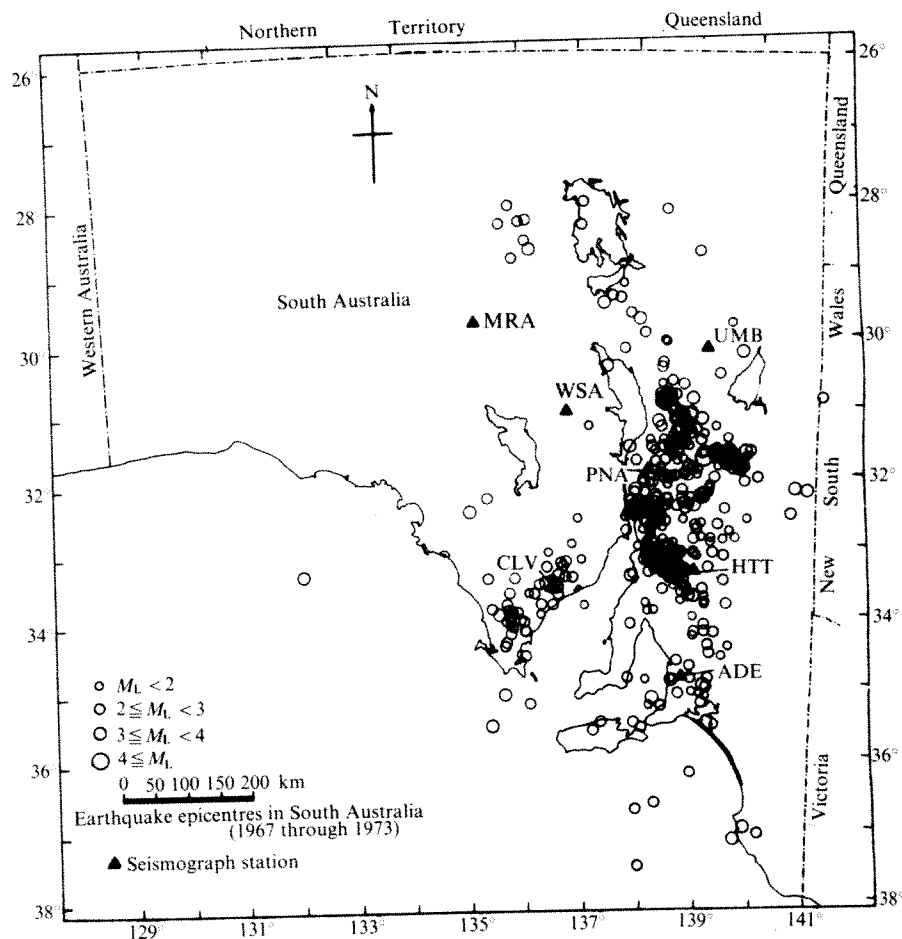


Fig. 1 South Australian seismicity, 1967–73.

Mechanism studies⁴ strongly suggest that right-lateral movement occurs between the eastern and western sections of the system, unlike the left-lateral shear originally proposed by Cleary and Simpson² and quoted by Coward¹. The right-lateral movement may continue southward along the transform fault to the mid-ocean ridge. This sense of motion on the north-western extension

oscillatory⁴, perhaps because of variations in plate velocity across Australia. The postulated left-lateral movement of 150 km (ref. 1) may be large in view of the remarkable uniformity of crustal thickness over much of the Adelaide Rift Zone¹¹. Although this may have been possible if faulting was almost pure strike-slip (as on the northwestern extension of the rift), smaller relative

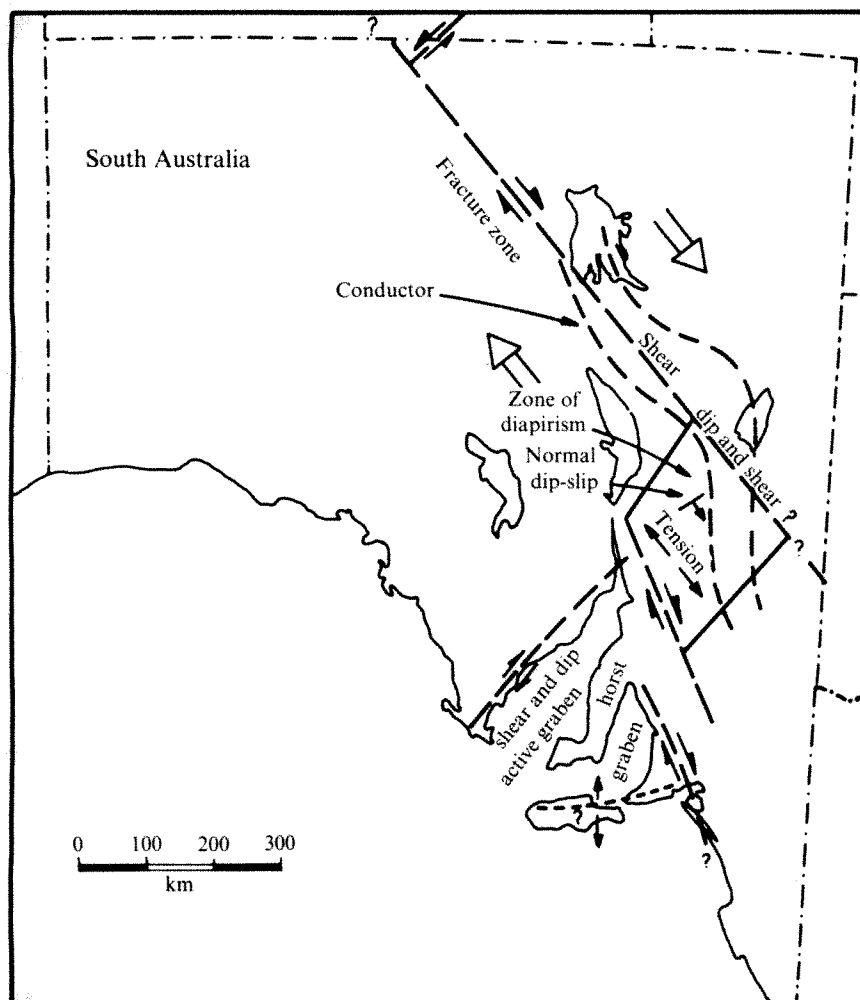


Fig. 2 Tectonic model for South Australia, showing the present sense of movement.

movements of only a few tens of kilometres may have been sufficient to produce vertical movements of several kilometres and much of the known geology of the region⁴.

Right-lateral shear may have been as important as left-lateral movement in the Adelaide Rift Zone, while dip-slip faulting may have been prominent in parts of the geosyncline. An interpretation of fold patterns should therefore allow for possible variations in the orientation, style and sense of faulting. The extent of shear movement across the whole system may also need revision.

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Large scale Palaeozoic shear zone in Australia

HYPOTHESES invoking large scale horizontal displacements¹, fracture zones and the movement of plates², then ideas rejecting 'megashield'³, and now a revival of the large scale Palaeozoic shear zone concept⁴ have been generated to explain the pattern of geological structures observed in the Adelaide geosyncline of South Australia. The latest attempt⁴ has curiously ignored reference to earlier discussions¹⁻³ which I believe are strongly relevant to the basic argument of whether a Palaeozoic shear zone is a tenable hypothesis. It would seem that the whole spectrum of concepts has now been covered; sinistral movements⁴, dextral movements¹ and no relative movement³. Although Coward's case⁴ is rather difficult to assess, the idea seems to have appealed to Sutton⁵ who cited Coward's model as a possible example of deformation within continental plates.

Crawford and Campbell's model¹ supposed that, "The Adelaide geosyncline originally extended meridionally along the line of the present Flinders Ranges . . .", whereas on Coward's model⁴, "a regional pattern of folds on north-east-south-west axial planes was modified by a sinistral shear couple . . .".

Palaeomagnetic evidence from the Precambrian of South Australia was cited⁶ to disprove the extension of the Crawford-Campbell model to involve regions of the Gawler Block; this has since been acknowledged⁷. We made it quite clear, however, that the data obtained earlier from the southern part of the Adelaide geosyncline⁸ were inadequate (because of metamorphic effects), to permit comment on whether that region had suffered 'rotation' from shearing. The only region of the Adelaide geosyncline to yield acceptable palaeomagnetic data was the central region of the Flinders Ranges⁹. Those data, by comparison with late Precambrian-Ordovician data from Western Australia, central and northern Australia, are consistent with the principal axis of the geosyncline having maintained a meridional trend. The data are not consistent with originally north-east-south-west trending axial planes.

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Matters arising

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obituary

Keith Bullen came to Cambridge from New Zealand, and entered St. John's College in 1931. It was his great good fortune there to meet Harold Jeffreys, who was just embarking on a task which was to be momentous for the development of seismology. This was the construction of new travel-time tables for earthquake pulses, using sophisticated statistical techniques and taking full advantage of the data collected over several years in the International Seismological Summary. Jeffreys asked Bullen to assist him. In spite of the very irregular distribution of seismological stations over the Earth's surface and the inaccuracies of instruments at that time, the Jeffreys-Bullen Tables (1935 and 1940) have endured astonishingly, and are still in common use.

In 1936, using the distributions of seismic velocities with depth which were derived by inverting the travel times, Bullen obtained his first model of the distribution of density throughout the Earth—and for the rest of his life he was deeply involved in successive efforts to improve density models. He told the story of his work with skill and enthusiasm in his book *The Earth's Density*, which was published in 1975.

Bullen explored the density distribution in two ways. The seismic velocities alone are not sufficient to determine the two elastic constants and the density. One further physical assumption is

needed. In the series of models called *Bullen A* he used a relationship between pressure, density and compressibility following the method of Adams and Williamson, whereas in the series *Bullen B* he assumed that compressibility and the rate of change of compressibility with regard to pressure varied smoothly throughout most of the Earth's interior. The results of the two different methods were largely reconciled when satellite observations produced a significant correction in the accepted value of the Earth's moment of inertia.

After 1960, when observations of the periods of the free oscillations of the Earth became available, Earth models could be revised so as to fit these also. Bullen and Hadden produced a new series of models designed to do that, but Bullen noted that they had not been able to allow for the effect of anelasticity, which, as Jeffreys had pointed out, tended to lengthen the periods of free oscillations.

His experiences in trying to compare Earth models put forward by different workers convinced Bullen of the need for a single reference model with which all variants could be compared. He became Chairman of the Committee for a Standard Earth Model, sponsored by the I.U.G.G., and worked hard for it until ill health intervened.

Bullen wrote an *Introduction to Seismology* which is uneven in its demands on the reader, but has run

into three editions and is still found very valuable. He also wrote an excellent *Introduction to Dynamics*, now in its eighth edition. A great asset of the book is Bullen's effort to give the reader insight into the reasons which determine the next step to be taken, and to explain why certain methods succeed and others fail. Throughout the book one finds, under the heading S.P.I.C.E. (special point(s) in choosing examples) the type of helpful suggestions that a good tutor would make to a pupil.

Bullen was a brilliantly clear expositor, whether writing or lecturing. He was also a very effective and strong (sometimes obstinate) committee man, so that he was called to many positions of responsibility, especially on Australian scientific bodies and in the I.U.G.G. and the International Association of Seismology and Physics of the Earth's Interior. His outstanding contributions to seismology were widely appreciated, and many honours fell to him, including Fellowship of the Royal Society in 1949. He was awarded the Gold Medal of the Royal Astronomical Society, and medals of several Australian and American scientific societies.

Bullen was a man of great energy and resource, in spite of partial deafness throughout much of his working life; he travelled very widely. He died on September 23, 1976, aged 70, leaving colleagues and friends in every continent.

E. R. Lapwood

announcements

Meetings

November 19, **Magnetospheric Dynamics**, London (Royal Astronomical Society, Scientific Societies Lecture Theatre, 23 Savile Row, London W1, UK).

November 23–26, **Future of Aircraft All-Weather Forecasting**, London (The Manager, Conference Department, Institution of Electrical Engineers, Savoy Place, London WC2R 0BL, UK).

December 6–10, **Fall Meeting of the American Geophysical Union**, San Francisco (Meetings Registration, American Geophysical Union, 1909 K Street, N.W., Washington, D.C. 20006).

December 9–10, **Technologies for Rural Health**, London (The Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, UK).

March 30–April 1, **Mixing**, Cambridge (The Organising Secretary, 2nd Mixing Conference, BHRA Fluid Engineering, Cranfield, Bedford MK43 0AJ, UK).

September 13–15, **Particle Size Analysis**, Salford (Dr D. Dollimore, Department of Physical Chemistry, University of Salford, Salford M5 4WT, UK).

October 25–28, **Radar**, London (Deadline for abstracts: January 10) (IEE Conference Department, Savoy Place, London WC2R 0BL, UK).

November 22–24, 1977, **Design and Applications of EHV Substations**, London (Deadline for abstracts: November 29) (IEE Conference Department, Savoy Place, London WC2R 0BL, UK).

April 16–20, 1978, **Luminescence**, Salford (D. Irish, Pye Unicam Ltd., York Street, Cambridge CB1 2PX, UK).

September 4–7, 1978, **Medicinal Chemistry**, Brighton, Sussex (Symposium Secretariat, VIth International Symposium on Medicinal Chemistry, 31 Plane Tree Way, Woodstock, Oxford OX7 1PE, UK).

September 18–22, 1978, **Fat Research**, Brighton, Sussex (The Assistant Secretary, S.C.I., 14 Belgrave Square, London SW1X 8PS, UK).

nature

November 18, 1976

Don't move!

FOR many years commissions, committees, working parties and individuals have pondered the problem of the scientific civil servant and his or her career prospects. In the last year or two much of this thinking has been fuelled by concern (at least by those on the outside) that the civil servant, with a generally securer job and an excellent pension scheme, has also been paid considerably better than his counterpart elsewhere. But long before this became an issue many thinking people, by no means all outsiders, were worried that the scientific civil service lacked either enough right openings for mature scientists or any means of goading such scientists not to sit and fester within the service. The Bondi Task Force which reported in 1974 was the most recent group to look at the problem, and came up with proposals to increase mobility into and out of the service.

A small unit was set up in the Civil Service Department in 1972, initially to work with the Bondi Task Force but eventually to proceed under its own steam in stimulating mobility. Unfortunately, its terms of reference were limited. Many believe that the best way to stimulate a scientist to give of his best and the best way to encourage an organisation to make the best use of its manpower is for the employer to make it quite clear to the employee at the outset that no one can be promised forty years of continuous satisfying employment. Under these circumstances there could be a valuable trade at all levels of people into and out of the civil service.

But this is not to be—and at times like the present, when projects are being trimmed or cancelled, note how the civil and public servants remain while industrial contractors have to cope with redundancies. The unit which was established was not given the job of helping people to make major switches in career; it was given the much more timid mission of stimulating two-year (typically) secondments into and out of the service—but with a guaranteed return ticket. And even this relatively unadventurous project has hardly met with waves of enthusiasm, as a recently circulated progress report of the Scientists Interchange Unit makes clear.

It is difficult to estimate exactly how many within the service might in one circumstance or another be candidates for secondment, or on the other hand how many

openings might arise which could be filled on a short-term basis by outsiders. But there are 2,300 Principal Scientific Officers in the service, and if we include other grades above and below this level and some in the analogous technical officer grades there might be 5,000 who should at least know about the scheme.

Yet in the last three years only 23 made an approach to the unit, and of these only five could be found places outside—four in industry, one in a research council. In the same period 20 secondments were made into the service—nine from industry, ten from universities and one from a research council. It is only fair to add that government departments sometimes arrange their own secondments without going through the unit, and in the same three years 18 did an outward move (though less than half into industry) and nine an inward move (two from industry).

The picture, as the unit admits, is disappointing. There seems little enthusiasm for tasting another job even with all the safety of one's own job being kept warm. It would be easy to conclude that the average British scientist is feeble-minded and unenterprising. That may indeed be true, but it should be made clear that the present scheme is actually a much more tricky one to make successful, and maybe a much less attractive one than a one-way ticket scheme. Skills have to be closely matched, employers have to be convinced that loss of employees for two years is in their interests, petty jealousies over salaries have to be overcome, temporary accommodation and schooling has to be found if a move is necessary, the briefing period may be a significant fraction of the total time available and so on.

Permanent moves could not be more difficult and indeed might be very much easier for all concerned. Certainly without them the same old accusations will continue to be thrown at the civil service, and scientists will have achieved little on their side to facilitate the more open style of government for which many yearn and which requires more flow into and out of government establishments. The Bondi Task Force should reconvene to consider, even in a time of gloomy economic prognostications, whether it cannot push for more than just secondments. □

Nuclear power, public interest and the professional

In the case of major and complex technological decisions in our time, such as that on nuclear power, it is sometimes said that the public is unable to make a full evaluation on its own and needs to trust some established professional and political mechanism instead. Jan M. Döderlein of the Institutt for Atomenergi, Norway, comments on the roles of the professionals and the nuclear critics.

NUCLEAR technology came to maturity at a time when attention was rightly focused more than before on the environmental aspects of industrial and energy production. As a result, in many countries nuclear stations are the only type of electric power plant for which detailed safety and environmental evaluations by government agencies are required, and where these evaluations are open to direct public participation. This has helped to create a public controversy over nuclear power, a controversy which appears in large measure to have spread from the United States. It presently focuses on three main topics: nuclear reactor accidents, the handling of radioactive waste, and the increased risks of proliferation of nuclear weapons (and therefore presumably of nuclear wars) from an expanding peaceful use of nuclear power.

Probably no technological decision in the history of mankind has been the subject of so many detailed studies, so much open discussion and such broad public participation. Amongst it all, some nuclear critics have given prominence to ethical aspects of the nuclear decision. This is justifiable to a certain extent since the application of nuclear power—indeed of any power—raises ethical questions. To carry this view to extremes in the way it is sometimes done, however, represents a vain attempt to set aside fundamental laws of nature and society.

One aspect of such ethical considerations is seldom if ever mentioned. The number of publications on astrology, on transcendental meditation, on nirvana and similar subjects is increasing sharply. Among the younger generations, and in some leading cultural and political groups, increasing numbers of people are seeking mystical or irrational escapes from many realities of modern society. There are thus clear indications that superstition, mysticism and emotionalism are on the upsurge.

Another symptom of this surge of emotionalism is the apparent tendency among sensitive and highly responsible citizens to shoulder the burden of guilt associated with the expansion of modern society. With some leading personalities in politics, industry and science, this burden is transformed into a desire to steer the development of

society away from perceived future cataclysms. Sometimes the bases for assuming such cataclysms are world models created by scientists. These provide some predictions of a near disastrous future for our society with the combined authority of science and leading personalities in society. Such an authority is merited neither by the quality of the world models nor by their basic assumptions.

One of the consequences of irrationalism and emotionalism is a desire for simplification of life and for a low energy society, a desire which is reinforced by "scientific" doomsday prophecies. Another is a strong sentiment against the people who are working for the application of technology within society, which erodes confidence in expertise. Nuclear power is not a simple technology; it can produce prodigious amounts of energy; emotionally it can be construed as having a certain "doomsday character"; and it requires qualified expertise. Nuclear power can therefore be seen as the antithesis of irrationalism, a view which easily creates a strong, emotionally based anti-nuclear attitude.

A natural outlet

The nuclear controversy is therefore a natural outlet for many irrational needs in our society, not least important of which are the irrational needs of many scientists, technologists and experts. These views are supported and broadened by fundamental observations made in another context by the epistemologist Karl Popper. He stated that, when threatened,

"the rationalist attitude to social and economic questions could hardly resist when historicist prophecy and oracular irrationalism made a frontal attack on it. This is why the conflict between rationalism and irrationalism has become the most important intellectual and perhaps even moral issue of our time".

One perspective on the nuclear debate, then, is that our dominant problem may be that of fighting emotionalism and irrationalism. This is not to use the words "irrational" and "emotional" in any derogatory sense. The fight is not against emotions or the irrational. The issue is the application of irrationalism and emotionalism in attacking major problems of our real, physical world.

The emotional and ethical aspects of nuclear power are trumpeted particularly by two groups of critics having the common denominator of emotionalism. One group consists of people for whom nuclear power is a vehicle of self-realisation. They play out some of their inner and emotional needs by taking an active part in the nuclear controversy, and the factual nuclear issues seem to play a secondary role. The other emotional group of nuclear critics is composed of professional and amateur politicians, notably leftist groups, anarchists, some populists and some environmentalists. Nuclear questions, real or imagined, are a means by which they further their own political goals, sometimes clandestinely, sometimes openly. A very different group of critics, the opportunists and malcontents, is cynically achieving fame and prominence by going into the nuclear debate, writing in the newspapers, giving lectures, going on television and so on. Needless to say, similar people are also found in the so-called nuclear community.

Characteristic shared

All three groups of nuclear critics share one characteristic: they seem to accept authoritarianism as a road to knowledge, often to the exclusion of other sources of knowledge. They rarely support their assertions with reasoned arguments or with facts, and they consistently invoke the opinions of some distinguished scientists, some Nobel-prize winners or some professional societies, ignoring others who disagree with their views. By confusing technical facts which can never be argued qualitatively, with value-judgments which can always be argued, the critics have in some countries succeeded in misleading sections of the public, causing them to believe that everything is open to argument and that the experts are confused. The truth is that the experts are not confused in their own field of expertise. Outside their own field they are not experts.

In the past two years we have seen a plethora of manifestos and statements for or against nuclear power by Nobel prize winners, by groups of scientists and by various professional societies. In connection with the statements, the eminent or special qualifications of the participants are always emphasised. While possibly not without merit, such statements should be viewed very critically. Practically without exception the participants in the anti-nuclear manifestos have no technical background in fields relevant to nuclear technology. Against the participants in pro-nuclear

manifestos, this criticism is less relevant.

One criticism is valid against all manifestos on nuclear power. The scale and timing of the introduction of nuclear power is a major and complex decision, resting on technical, economical, political and other social considerations. In a democratic society such decisions are not left to technological experts but to elected political officials. A professional using his specialist background in support of personal views on social questions tries to carry his professional authority over into fields where he should no longer have such authority, into fields where his opinions should be weighted on the "one man, one vote" principle.

Members of some professions believe they have more specialised knowledge, and that their political conclusions are based on sounder analysis of the evidence than the public. If one expects the public to have confidence in the role of professionals in decisions, the opinions of highly regarded experts within their chosen profession must be accorded a certain authority. But it is equally important not to support a carry-over of professional authority to political and ethical questions, a carry-over which may indicate a certain intellectual arrogance.

Assessing the 'facts'

Many "facts" in the nuclear controversy are to all appearances hotly contested. Some of these "facts" are not really facts, and deserve to be independently and critically evaluated by professionals and non-professionals alike. Among such "facts" in the nuclear debate are some aspects concerning the long term effects of radioactive waste, and the possible connections between nuclear power and proliferation of nuclear weapons.

Among the often repeated and confusing statements on radioactive waste are the suggestions that in this waste man produces materials that (uniquely) remain toxic for times much longer than we have had experience with, and that in nuclear reactors we have created materials that have never existed before.

Certain elements created in substantial quantities by nuclear power, for example plutonium (but not waste proper), remain toxic for times longer than the period of human civilisation. No industrial scale method for disposing permanently of radioactive waste is in use today. Deciding on appropriate handling of this waste is an important and difficult question which many countries are addressing. At a pilot plant level, however, there are solutions to the *technological* problems. Moreover the long life of

radioactive waste should not be judged in isolation; industry including power production from oil and coal gives uncontrolled releases to the environment of substances which remain toxic for infinite times; civilisation is accustomed to living with poisonous materials that remain toxic for times longer than the life of radioactive substances. For civilisation as we know it there is thus only a choice of *which* toxic materials we wish to handle.

With nuclear power man has not created any materials new to earth. Specifically, fission products and plutonium are naturally occurring elements, albeit in small concentrations. The ethical objection to nuclear power sometimes made on the basis of creation of "new" elements therefore has no foundation in fact.

The question of a possible connection between nuclear power and proliferation of nuclear weapons really has two separate aspects: one concerning sub-national groups using atomic explosives for terrorist purposes, the other concerning a possible increase in the number of sovereign nations with access to nuclear weapons. The understandable but unfortunate preoccupation of the mass media and the public with the matter of nuclear terrorism is distracting our attention from the far more important question of possible proliferation of atomic weapons beyond the present weapons states.

It is misleading to ask whether the spread of nuclear power inevitably leads to the spread of nuclear weapons. The peaceful uses of nuclear technology are now widespread; there are substantial quantities of peaceful and military fissile materials. The relevant question is whether a halt to the expansion of nuclear power, or to all use of nuclear power, will substantially reduce the potential for wars and the risk to the public from wars. Clearly it would not be meaningful to curtail the use of nuclear power for this reason without at the same time dismantling the many smaller reactors used for medical purposes, isotope production and research in most countries of the world. This aspect takes on added importance when one considers the substantially more complex operations required for using a power station rather than a small reactor for plutonium production.

Technological developments are likely to complicate further our questions about weapons proliferation. Within a few years there will be available, worldwide, several new techniques for enriching uranium. All of those being researched today are potentially simpler technologically than the present diffusion technique, and all of them can more easily be applied on a small scale. Ensuring peaceful uses

only of these techniques may be of more importance than the question of plutonium safeguards.

Unfortunately, nuclear proliferation is only part of a more fundamental and more serious contemporary question: can anything short of actual war stop a determined sovereign nation from getting primitive but usable versions of any kind of contemporary weapons technology—nuclear, bacterial, chemical or any other? Be that as it may, broad agreement could probably be obtained to curtail development of nuclear power if rational arguments could support an assertion that stopping peaceful uses of nuclear energy would reduce, if only marginally, the risk to the public from war and acts of terrorism. But reasonable, rational and valid arguments to this effect have not been put forward so far.

Reducing the risks

On this basis, expending efforts to stop the use of nuclear power does not seem to be a credible and useful way of reducing the risks of nuclear wars. Our most responsible and efficient attack on the nuclear proliferation problem would seem to be to channel our efforts into support for the UN and the nuclear supplier nations in their non-proliferation efforts; into the expansion of international safeguards inspection, including inspection of arrangements to protect physically all fissile material; into support for all practicable means of making the time span and the effort involved in converting in-reactor plutonium production to a workable nuclear weapon as large as possible; and last but not least into the problems created by the availability of new uranium enrichment techniques.

All human actions entail some unforeseeable consequences. Plans for action cannot be based solely on factual proofs and logical deductions; they must finally rest on political decisions made in the face of uncertainty, a point which gives a perspective on the place of technological facts in a world of values and emotions. Clearly, a number of irrational, emotional and ethical factors may be and should be of importance in a choice of power plants for the production of electricity. But an evaluation of *rational* and *quantifiable* factors tells us how many lives, which environmental improvements and what economic advantages we have to sacrifice in order to satisfy such emotional demands. In defining the role of the professional and in protecting the public interest, the importance of emotional factors in the nuclear controversy must be admitted; but as many problems as possible should be decided on a reasoned, factual and rational basis. □

No university without research

Gillian Boucher looks at how the Open University copes with the problem of conducting research

IT would be a forgivable mistake for anyone outside Britain's Open University to imagine that its sole function was to provide a university education by post: virtually all publicity has concentrated on its innovative and immensely popular courses. Even within the university it has sometimes been difficult to remember that there could be any aim in life beyond producing the next course unit. But the Open University's charter is utterly normal in allowing and requiring staff to undertake research, and recently, with the worst of the growing pains over, there has been a reassertion of the vital need for research to take its proper place alongside teaching.

Of course this view has always existed: most open university lecturers like research too much to forego it willingly and believe that an institution that simply packages information does not deserve to be called a university. But initially many people just did not have time for research. There are some who have gravitated towards the Open University because they get more satisfaction from teaching than from research. And there is a strong current of opinion in the university administration that says that research is a prize for the good boys who have written their courses. In some faculties there is even a feeling that science in general and scientific research in particular are expensive luxuries. But in spite of the difficulties the university has never been devoid of research.

Steven Rose and Ian Gass, professors respectively of Biology and Earth Sciences, managed to bring a nucleus of staff with them when they moved to Milton Keynes and have been gradually stepping up their activity ever since. The Department of Earth Sciences now produces more papers than any other British Earth Sciences Department except the Cambridge Department of Geophysics. The small staff (10 lecturers in the Biology Department) means that it is not feasible for everybody to work independently: intellectual companionship is only gained by joining a more or less interdisciplinary group. The obvious example is Rose's Brain Research Group, which involves five lectures and 15 others and is by far the largest research effort in the Biology Department. Another group, the Energy Group, was formed spontaneously during the preparation of a course on materials. Now the scientists, engineers, architects and economists in the group are studying

problems as various as the British gas industry and the development of a house heating system based on the same principles as the refrigerator cooling system.

Funding a problem

One of the programme's great advantages, of course, is cheapness. Funding is obviously a problem for an institution trying to grow at a time of economic crisis and government lack of sympathy with the universities. For an established group funding from the university and from outside sources is adequate if not princely. But for others there are difficulties: the university's approach is to fund a project initially but to expect it to attract outside funds to continue if it is any good; if the strings of published papers required by the SRC have not been written, the project is likely to founder.

Working conditions are not very comfortable and one of the problems in creating a stimulating academic environment is that people tend to spend a lot of their time at home writing course units. The permanent science building originally scheduled for 1974 will be ready in 1977 at the earliest and even this will not hold all staff: the biophysics and other groups temporarily housed in Oxford will stay there for at least another five years. That is not an unmitigated disaster—among the charms that Oxford possesses and Milton Keynes lacks are first-rate libraries and the ease with which temporary self-funded American

researchers may be attracted.

The Open University has always had research students—both internal students at Milton Keynes and Oxford and external students for whom the university finds an external supervisor near home. Many external students are technicians or hospital scientists and must of course have access to the equipment they need. Trying to do a PhD and a job at the same time sometimes causes insuperable problems: in many areas knowledge is increasing almost too fast for a part-time researcher to keep up, let alone make his own contribution.

At present higher degree students with Open University first degrees are rare, simply because so few Open University graduates have yet been produced. When they do start demanding higher degrees in large numbers there will be difficulties. The open university student, though probably more resourceful than the average British undergraduate, is not used to reading much outside his course text or doing much practical work. In many ways the Open University degree is closer to the American than to the conventional British one. American PhD students usually spend a couple of years doing course work before beginning research but the Open University offers no postgraduate courses. The prospects are not good: the undergraduate courses are voracious and the Department of Education and Science has not responded warmly to the suggestion that it might earmark certain sums for postgraduate courses. In terms of mere numbers this is understandable—at present there are 50,000 undergraduates and 400 graduate students—but the development of postgraduate courses will be valuable. □

Profits in academia

One way the Open University is hoping to cushion itself from the uncertainties of government funding is by setting up a company to market its educational materials. Marketing as a part of the university administration is as old as the university itself but it is hoped that Open University Educational Enterprises Ltd will greatly expand the business.

All is still in the air, though, while the university discusses the project with the Department of Education and Science which, after the resounding failure of a similar venture, British Museum Publications Ltd, is somewhat nervous of letting public funds be used as risk capital. The OU, however, is confident that the go-ahead will come in time for the company to start business on January 1 as planned. Large amounts of capital are not required as some of the present marketing division's profits are ploughed back into marketing. According to the university the talks are mainly to thrash out the right relationship of the new company to the university and to make sure that it is properly structured.

From the university's point of view, a company owned by the university has several advantages over a division of the university administration, among them the freedom the company would have to offer salesmen a suitably attractive salary instead of having to pay them on academic administration scales. The company may also clarify the copyright position—at present although the written word is the main teaching medium in the Open University the academics hold the copyright on their works as in any other university.

More than half the income from the sale of the Open University's books, tapes, films and cheap scientific equipment comes from abroad. The university feels there is plenty of room for the marketing company to expand: the marketing division cannot deal as well as it would like even with its present markets. If the new company succeeds, as is hoped, in increasing turnover from the present £700,000 a year to £1 million by 1980, it will be contributing a significant supplement to the £12 million government grant.

USA

Seeking Senate surgery

A radical overhaul of the Senate Committee system is being discussed in Washington. Colin Norman reports on the implications for scientific affairs

AN entertaining display of political horsetrading, perhaps culminating in a good public scrap, is about to unfold on Capitol Hill. For the first time in more than a quarter of a century members of the US Senate are being forced, somewhat reluctantly, to take a close look at that august body's confused and inefficient but immensely important system of legislative committees. Some sweeping reforms, which could wipe out entire committees and with them the power bases of a number of influential Senators, are under discussion.

The proposed reforms were put forward a few weeks ago by a special select committee. But because public attention was diverted by the marginally more interesting spectacle of the Presidential election and a third of the Senate were preoccupied with getting themselves elected, few people took much notice. The Senate is expected to take up the proposals soon after it reconvenes in January, however, and so they are now becoming a topic of intense interest on Capitol Hill.

There are a good many political obstacles to be overcome before the proposed reforms are accepted, but if they are ever implemented they will have a substantial effect on the way the Senate handles legislation on scientific and technological matters. For a start, responsibility for energy research and development would be placed in a single committee, the jurisdiction of the present Commerce Committee would be expanded to take in most non-military science and technology programmes, and the Joint Committee on Atomic Energy, which has charted the course of the United States' nuclear policy for some 30 years, would be abolished.

Overall, the proposals would reduce the numbers of Senate committees from 31 to 15, doing away with a variety of select, standing and joint committees, and realigning the jurisdictions of most of the remainder. The Senate's present 174 subcommittees would be cut down to about 100, and no Senator would be able to sit on more than 8 subcommittees. In short, the proposed reforms represent some pretty radical surgery, and they would bring about the first real changes in the Senate committee system for 30 years.

Even a cursory look at the system confirms the need for a knife. The Senate's committees are so sprawling

and their jurisdictions so confused that it is frequently difficult to tell which committee has authority over individual bills. Take energy research and development, for example. The Joint Committee is deep into one aspect of energy, so is the Interior Committee, the Public Works Committee, the Aeronautical and Space Science Committee, the Commerce Committee and the Labor and Public Welfare Committee. A couple of years ago, when a relatively non-controversial solar energy bill was passed by the House, no fewer than four Senate Committees claimed jurisdiction and it had to be approved by all of them before finally reaching the Senate floor.

But, though the need for reform is obvious, it is going to be a tough battle to get anything done. The problem is that Congressional committees, and to a lesser extent subcommittees, represent overlapping fiefdoms headed by powerful, and frequently elderly, chairmen whose authority has taken years to acquire. The chairmanship usually goes to the longest-serving member of the majority party on each committee, and with it comes a good deal of power to appoint committee staff, and to shape or even obstruct the passage of legislation. Thus any attempt at reform must first overcome the fact that a lot of powerful people are going to resist having their authority altered or even removed.

The House went through a mild reorganisation a couple of years ago in a move which gave the House Committee on Science and Technology a good deal more power. But those changes were only made after a couple of years' of intense negotiations and six days of shrill public debate. The Senate reforms may be equally difficult—moves are already afoot to try to shunt them aside by simply referring the whole matter to the Senate Rules Committee for further, prolonged study—but their proponents point out that conditions for change are more favourable now than at any time in the past several years. For one thing, there will be seventeen new faces in the Senate next January—a much higher turnover than usual. And for another, the chairmen of four committees threatened with the axe were either defeated or retired.

The following are the major changes proposed by the select committee in committees concerned with science and technology. It should be noted that the proposals would leave essentially untouched the real powerhouses, such as the Appropriations Committee, the

Armed Services Committee, the Finance Committee and the Judiciary Committee. Thus, some of the most powerful Senators won't be too badly affected, and appropriations for government agencies will continue to be handled by the same appropriations committees and subcommittees.

- It has been proposed that responsibility for non-military research and development policy should now fall under the present Commerce Committee, whose authority would be greatly expanded and whose name would be changed to the Committee on Commerce, Science and Transportation. The new committee would be responsible for keeping its eye on the activities of agencies such as the National Science Foundation, the Office of Science and Technology Policy, the National Aeronautics and Space Administration and the various agencies of the Departments of Commerce and Transportation. It would be the chief committee for federal research activities other than military, biomedical and energy programmes. If the proposed changes are accepted, they would cause the demise of the Aeronautical and Space Science Committee whose chairman, Frank Moss, was defeated at the polls in Utah.

- The change would also mean that Senator Kennedy would lose the authority he now holds over the National Science Foundation through his chairmanship of the NSF subcommittee of the Committee on Labor and Public Welfare. That committee would be expanded under the proposed reforms to become a Committee on Human Resources, and Kennedy is said to be negotiating to have NSF affairs consigned to it. It wouldn't be the most logical place for NSF, but at least Kennedy would be able to retain his jurisdiction.

The proposed Human Resources Committee would be responsible for, among other things, health and biomedical research, which means that Kennedy would retain much of his authority over the programmes of the National Institutes of Health.

- As for energy research and development, the select committee has proposed that the responsibilities of the present Interior Committee, headed by Senator Henry Jackson, should be expanded to include most federal energy development efforts. Renamed the Committee on Energy and Natural Resources, the new body would pick up responsibilities from the Joint Committee on Atomic Energy and a number of other committees. The proposal would put the knife into the back of the Joint Committee, the once-powerful unit which has shaped nuclear policy

since the second World War.

The Joint Committee is in trouble for other reasons, too. Next month, when Democratic members of the House meet to discuss the political agenda there, two nuclear critics, Clarence Long of Maryland and Jonathan Bingham of New York, will propose that the committee be scrapped and that some of its functions be transferred to the House Science and Technology Committee, among others. Thus, the Joint Committee is under combined attack in both the House and the Senate, and its chances of surviving are generally considered to be less than even. One point going against the committee is that five of its nine members, including the chairman, Senator John Pastore of Rhode Island, either retire at the end of this year or were defeated at the polls. Moreover, two of the most powerful members of the Joint Committee, Senator Jackson and Representative Mike McCormack, would stand to inherit some of its

authority in their own committees, so they probably wouldn't be too sorry to see the Joint Committee disappear. It should be noted that the Joint Committee has been consistently pro-nuclear, and its demise would be welcomed by anti-nuclear critics.

● Another major Senate change proposed by the select committee is the creation of a new committee on Environment and Public Works, founded mostly on the present Public Works Committee headed by Senator Jennings Randolph. It would be responsible for environmental programmes together with nuclear regulation, which would mean that responsibilities for developing and regulating nuclear power would be divided between separate committees.

The proposed reforms are expected to be one of the first items of business when the Senate reconvenes early in January, but negotiations are already under way behind the scenes, as Senators who stand to lose some of

their jurisdiction are striving to hang on to their authority.

It is quite possible that the opposition will be too strong and that the proposals will be quietly killed off or severely watered down by consigning them to the Rules Committee for further study. But at least the proponents of the reforms are preparing to put up a spirited fight. Senator Adlai Stevenson, the chairman of the select committee, is said to be threatening to introduce a resolution blocking the appointment of new senators to committees and the appointment of new committee chairmen if his proposals are simply shunted aside. Such a resolution could tie up the business of the Senate for a long time if it were approved. A more likely development is that differences will be settled by behind-the-scenes negotiations in the next few weeks, and a compromise reform plan will be worked out in conjunction with the Rules Committee. □

Genetic manipulation: enter the environmentalists

LAWYERS for two environmental organisations have filed a formal petition with the Department of Health, Education and Welfare (HEW) requesting extensive public hearings and the development of legally binding regulations to control all recombinant DNA experiments in the United States. It is the first time that environmentalist groups have formally entered the swirling dispute over the risks and benefits associated with recombinant DNA, and the petition is probably only the opening shot in what could develop into a lengthy legal fight.

Filed by the Environmental Defense Fund and the Natural Resources Defense Council, the petition has been endorsed by Robert L. Sinsheimer, chairman of the Division of Biology at California Institute of Technology. Sinsheimer, a leading critic of recently-issued safety guidelines governing recombinant DNA research supported by the National Institutes of Health (NIH), sent a letter to HEW along with the petition.

The petition requests two actions by HEW. First, public hearings should be held to allow interested parties to state their case and to have their views taken into account; and then HEW should develop binding regulations to control all recombinant DNA experiments in the United States. In the meantime, the petition asks HEW to extend the voluntary NIH guidelines to cover experiments supported by other agencies and by

non-government bodies.

The petition is clearly motivated by the belief that the NIH guidelines are inadequate to control the potential hazards associated with recombinant DNA research. It states that the guidelines "are the product of the deliberations of scientists who are now conducting recombinant DNA research", and argues that "little discussion was devoted to whether or not these experiments ought to be performed *at all*, even though the question was raised both by concerned laymen and by prominent scientists".

The petition also points out that the chief drawback in the guidelines is that they formally apply only to research supported by NIH, leaving industrial research essentially unregulated. Moreover, there is no requirement for federal monitoring to ensure that the guidelines are being followed. Those drawbacks are also bothering some state and local officials, in New York State for example.

The petition will probably be turned down on the grounds that HEW lacks authority to tell other agencies or private industry what to do, but the environmental groups are likely to press their case either in the courts or on Capitol Hill. Senator Edward Kennedy has already said that he may consider introducing legislation to make the NIH guidelines binding on everybody who wants to conduct recombinant DNA studies.

Meanwhile, an inter-agency task

force has been established in Washington to discuss ways in which the NIH guidelines can be extended to other federal agencies. According to one official concerned with the task force, it will also discuss the possibility of establishing a monitoring procedure to ensure that the guidelines are followed, and it will also look into what industry is doing and whether the federal government could (or should) regulate industrial recombinant DNA experiments. One possible outcome of the task force's deliberations is that the NIH guidelines may be made into legally binding regulations covering all agencies and industry as well. If so, that would accomplish some of the petition's demands.

The task force is headed by Dr Fredrickson, and consists of representatives of other federal agencies. It meets in private since it does not fall under the terms of the advisory committee act, and it is expected to come up with some recommendations by mid-January.

If it does recommend the adoption of legally binding regulations and a monitoring procedure, a key question would be which agency should take responsibility for enforcing them. NIH officials are anxious not to be placed in the position of both supporting and regulating the research, and the task may therefore fall to a regulatory agency such as the Occupational Safety and Health Administration.

USSR

Space programme: accentuating the cooperative

The Soviet Union will celebrate the twentieth anniversary of Sputnik-1 next year at the same time as the diamond jubilee of the October Revolution. Vera Rich reports

THE launching of Sputnik-1 on October 4, 1957, inaugurated a whole series of Soviet "firsts" in space, frequently co-ordinated (so far as the state of the art permitted) with some notable anniversary. The centenary of Lenin's birth was marked in 1971, for example, by the launch of the first orbiting space-station, Salyut 1; and it is entirely possible that the Soviet space programme is planning some equally prestigious "spectacular" for the sixtieth anniversary of the Revolution. The current emphasis, however, suggests that the year will be marked first and foremost by an extension of "fraternal cooperation" in space between the Soviet Union and her Comecon partners.

This trend is not a cover-up for the recent set-back to the Soviet space programme provided by the aborted Soyuz 23 mission when it failed to link up with the Salyut 5 station. On the contrary, it is perhaps best exemplified by the joint communique issued by Soviet Academician Boris Petrov and GDR Academician Karl Gröte, after the previous highly successful Soyuz flight. Soyuz 22 had carried a "multi-spectrum" camera from the Karl Zeiss (Jena) works, and was heralded as a new phase in Comecon cooperation in space. The same theme was enthusiastically repeated by the Soviet and Comecon media.

The presence of foreign equipment aboard a Soviet spacecraft is not new; French instruments were carried on Lunokhod 2 and by the Mars 5, 6, and 7 probes. Comecon has had its own space programme, Interkosmos, since 1967, and has launched 16 orbital satellites and 4 high altitude rockets. The Soviet Union supplies the rocketry for all Interkosmos launches, while her Comecon partners supply certain instruments, so the use of such instruments in a formally Soviet mission might seem a fairly predictable development. Why, then, was it greeted with such acclaim?

Propaganda plays a certain part: the current Comecon five-year plan, while temporarily soft-pedalling the final goal of total economic integration, is concentrating on a short term drive

(15-20 years) towards integration in certain significant areas, notably sophisticated instrumentation. The Petrov-Gröte communication clearly refers to this policy, stating that East German participation in Soyuz 22 was "part of the process of Socialist integration in one of the newest regions of contemporary science and technology".

The concept of an integrated science policy is, however, meeting with opposition from certain research bodies and institutions in the member countries of Comecon, particularly where, as in Poland, there is a long native scientific tradition. Such bodies have been openly accused of "autarchic" (that is, nationalist) tendencies, necessitating the intervention of Party and

Government. Emphasis on cooperation in such a prestigious field as space research might well be interpreted as simply a tactical measure to offset in the mind of the public the effect of this academic discontent.

Earlier this year it was announced that Interkosmos would start a manned space programme with multinational crews. The first launches are scheduled for 1977 and the cosmonauts are already in training. It would seem, however, that each crew is to include at least one Soviet cosmonaut. Since the Soyuz 11 tragedy, though, the Soyuz missions have used a two-man crew, instead of the previous three—a change apparently associated with the decision that the crew should wear space-suits during re-entry as a safety measure. If the current pattern continues, it will take at least eight flights for each country to get one representative in space.

Expeditions: resources rationally used?

The rational use of natural resources in the Soviet economy is a major target of the current five-year plan. It is also a dogma which can be invoked to justify most things. That includes geological and geographical expeditions, where the data obtained can be interpreted to relate to the needs of prospecting, oceanography, climatology and other fields of practical significance. In the case of the expeditions of summer 1976, the range is from the immediately practical to the extremely remote in terms of the economy's needs.

On the one hand, there was massive Soviet participation in the 200-strong joint Comecon expedition to Mongolia, which worked within a surveying programme aimed at ensuring 10-20 years' supply of fuel and material resources for the Comecon bloc. During this first field season some 7,000 km² was systematically surveyed, and several outcrops of "valuable raw materials" (so far unspecified) were located. The expedition is a long-term project; to facilitate its work in future seasons a special base is to be established next year on the outskirts of Ulan-Bator, the Mongolian capital, with administrative and living accommodation as well as such services as shops and a kindergarten.

Although even the most cautious planners could hardly doubt the practical value of the Mongolian expedi-

tion, they might feel a little different about the other major long-term project inaugurated this summer. This is the scheme to sink a 16-km borehole at Saatly in the Murganskaya steppe. This area is a "geological anomaly", where the crust is significantly thinner. In 1970, a pilot borehole was sunk to a depth of 6,240 m. If successful the new project will allow material to be recovered from the upper mantle. The theoretical value of such specimens would be immense; the most practical outcome of the expedition may well be the experience which the Soviet oil industry will gain in sophisticated drilling techniques.

One of the most practically-orientated expeditions this summer was that of the Siberian Energy Institute of the Soviet Academy of Sciences, which set out to survey possible power resources in the construction area of the new Baikal-Amur Mainline railway. The Baikal area has long been famous for its geographical and ecological riddles; this expedition discovered another, a complex of "classical mountain glaciers" at a relatively low altitude and unexpectedly far south. The plans for the railway have from the beginning incorporated a number of conservation measures aimed at preserving the unique Baikal habitat. The presence of these glaciers "in direct proximity of the line" is causing the relevant experts considerable concern.

The head of the cosmonaut training programme, Lieutenant General Vladimir Shatalov, has spoken in terms of a three-man crew, consisting of a Soviet commander and a flight engineer and research engineer from other Comecon countries. This suggests either a return to re-entry in shirt-sleeves or else the use of a larger spacecraft, and Shatalov has indicated that "in time" larger orbiting stations will be used, making it possible for the crews to include specialists from different fields. But he did not make it clear whether such stations would be in use by 1983, and present plans for Comecon participation go no further than this date.

It remains to be seen whether such joint missions form an integral feature of long term Soviet space planning, or whether, as in the case of the flight of the first (and only) woman cosmonaut Valentina Tereshkova, a policy hailed as a triumph of socialism may later be quietly phased out. The manned programme, however, is by no means the only new development of Interkosmos, nor in the long term is it the most significant.

Petrov and Gröte speak rather

vaguely of Comecon participation in "still more complex and interesting experiments". This may mean no more than the provision of equipment for Soviet spacecraft, a development that is virtually implicit in the "integration" policy which aims at increasing specialisation of the member countries. But Comecon is also to provide some of the ground facilities. Already the Comecon countries track the Soviet spacecraft. The accession of Cuba to Comecon provided an important new link in the network; new plans include the provision of various research facilities. A new astronomical observatory was recently opened in Prague as part of the Interkosmos programme.

Poland's contribution will be considerable—a Space Studies Centre within the Polish Academy of Sciences to include institutes of space physics and geodesy, earth and environmental resources, the application of space technologies, materials testing, and also a computer centre. According to Professor Stefan Piotrowski, a member of the Polish Academy of Sciences and also of the Interkosmos Council, the centre will work on methods of research into the effect of solar

activity on the ionosphere and atmosphere which, he says, will benefit communications, meteorology, agriculture and possibly medicine. The council will also draw up a programme of experiments to be carried out by manned spacecraft and satellites.

Although such facilities are designated part of the Interkosmos programme, it seems inevitable that the Soviet Union's own national space programme will also benefit from access to them; indeed, this would provide a further opportunity for "fraternal cooperation". However, certain joint Comecon projects have already evoked the criticism that the Soviet Union has made little contribution other than a site or the expertise, while the financial burden has fallen on her partners. However great the official acclaim of such cooperation, the possibility remains that those scientists already disenchanted with Comecon countries may view the new developments in the space programme not so much as a triumph for fraternal integration as a way for the Soviet Union to get routine research and development done at someone else's expense. □

NETHERLANDS

An unfavourable climate

Casper Schuurin reports on recent developments in science policy in Holland

THE Dutch Government is going to spend 2,560 million guilders (about £640 million) for research in 1977. Universities will use less than half (1,130 million), after a budget this year of 2,300 million. Industry plans to have 2,810 million guilders available next year for research and development after 2,510 million this year; at the end of last year industry had expected to spend 2,190 million in 1976, indicating that the climate for investment in research is not as favourable as it seemed. Some 390 guilders will be spent on research and development per head of the population.

These figures are part of the new budget presented to parliament by the science policy minister, Fokele Trip. He says science policy cannot be seen apart from the socio-economic development of the country: out of science policy a contribution should come to the selective growth of the economy which the government has outlined in the government memorandum on industrial policy issued earlier this year. Environmental factors, town and country plan-

ning and a policy to avoid the wasteful use of energy and resources all form part of that selective growth policy.

On the energy front 60 million guilders will be available for research as the specially formed fund for the sodium-cooled fast breeder reactor in Kalkar will be abolished. This will be used mainly for programmes other than in the nuclear field. It is surprising, however, that in the plans for research up to 1981 which were presented along with the science budget, the nuclear energy component will increase while the total energy research and development budget will hardly be more in 1981 than in 1977. The future for alternative energy sources is therefore looking very bright at the moment.

Mr Trip is also planning to spend more money for a popularisation of science. For five years the University of Amsterdam has organised courses for scientists to write articles for a larger audience beyond their own discipline, and it will now receive a government subsidy. Other universities are also starting such a course. At the School for Journalism a training-course for science writing will also be introduced with ministerial money, and the Royal Dutch Academy of Sciences is conducting a study on a science information unit to improve "science-



Trip: not available

society" communications; Dutch universities and some research institutes are, however, already more or less active in this respect.

The science budget received a favourable reception in the parliament's special commission on science policy. Mr Trip has said that for personal reasons he is not available for a government post after the elections in May 1977, and feels that a new minister for science policy is necessary. He says he will leave behind a sort of "last will" describing how best the position of such a minister can be used. □

IN BRIEF

Nuclear trade

Export of Australian uranium ore to the United States, Japan and West Germany, embargoed for four years, is to be increased but not matched by an extension of mining operations. Export permits are to be issued for Rio Tinto-Zinc to meet existing contracts, but exploitation of other mines must await the further findings of the Fox environmental enquiry.

Canadian sternness over the proposed French sale of a nuclear reprocessing plant to Pakistan has meanwhile resulted in France letting it be known that if Pakistan chose to cancel the contract no strong objections would be raised. The French have no intention of backing out themselves, in spite of modifications in their nuclear export policy acknowledging the dangers of nuclear proliferation. Canada provided Pakistan's only nuclear reactor, and has threatened to supply fuel for only

two years instead of six if Pakistan acquires a reprocessing plant.

● The informal association of nuclear exporters which met in London last week has grown to 15 with the addition of Switzerland, with observer status only. Members of the group have now been named officially. They are the United States, the Soviet Union, France, Britain, West Germany, Japan, Canada, Belgium, Sweden, The Netherlands, Italy, Switzerland, East Germany, Poland and Czechoslovakia.

Genetic proposals: reactions

The Institute of Biology has now expressed its concern about the UK Health and Safety Commission's proposals for regulating genetic manipulation experiments in Britain. Commenting last week on the Commission's consultative document, which was circulated at the same time as the Williams Committee report, the Insti-

tute says it is "neither practicable nor desirable" to have both a blanket regulation requiring advance notice to be given on all experiments and a clause exempting "what is likely to be over 90% of microbiological experiments".

The Commission, the Institute says, "has not available, nor will it be able to recruit, sufficient suitably qualified staff to deal with notifications and exemptions", and goes on to suggest that its involvement should be "solely as a body able to enforce recommendations" of GMAG, the advisory group recommended by Williams and now being established by the DES.

The Association of University Teachers (AUT) also made its views known last week. Making similar criticisms to those of the Institute of Biology, the AUT asks that the Commission think again "on the lines that it puts forward a much stricter definition of the experiments covered".

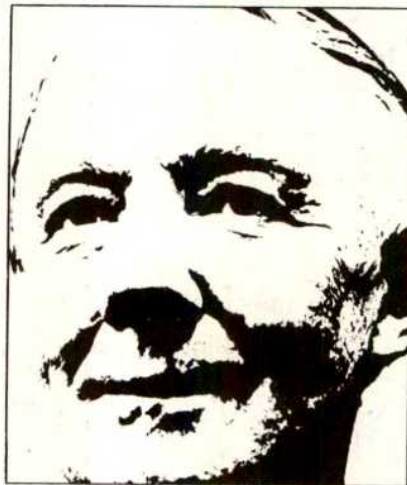
Our farm animals, as they exist today, have been produced by selective breeding from wild ancestors. Domestic cattle in Europe and North America grow rapidly when kept warm and dry with an ample supply of nutritious feed, and they give milk in large volumes under similar, unnatural conditions. Unless grazed on lush pastures of cultivated grasses whose productivity is maintained by the plentiful use of fertilisers, or unless they receive concentrated high-protein rations, their yield of meat and milk falls well below their potential. So long as the world produces a surplus of food to support this type of husbandry, the breeds which are at present the most numerous—the Friesian and the Charolais for instance—will retain their popularity.

As new and more productive types of cattle have been produced, so many of the older breeds have become less common, and some are already extinct. Similar processes have operated with sheep, pigs and poultry. The variety of the livestock on our farms is rapidly being reduced.

Most farmers have welcomed the simplification of having only a few well-characterised breeds of animals to choose from, and have not been worried by the disappearance of so many others. However, there has recently been more concern about the loss of so many breeds which have evolved during the last two thousand years, particularly as the original ancestors of the breeds cannot now be identified. Organisations such as the Rare Breeds Survival Trust have been established, and the results of

their efforts can be seen at the Cotswold Farm Park in England, and in Folk Museums in Scandinavia.

Many who wish to save our old breeds probably do so for senti-

Past breeder**KENNETH MELLANBY**

mental reasons, but they also justify their policy by suggesting that it has an educational value, to teach children and students about the history of farming. It is also claimed that some of the old breeds may be able to contribute valuable genes to future breeding programmes, and thus produce animals with virtues missing from existing commercial strains. Plant breeders have used ancestral forms of potatoes and cereals in this way, and animals might be treated in a similar manner. Unfortunately, though this may still be a possibility, most

breeders of cattle and sheep think that there is little valuable genetic material in the small surviving stocks of most old breeds. This opinion may be coloured by their preoccupation with animals suitable for intensive farming, where hardiness and the ability to survive are no longer sought.

I believe that we should look again at some old breeds as meat producers in their own right. As the world population grows, there will be less surplus food for intensive animal rearing. Our best land, in all countries, will be increasingly needed for crops for human consumption, and marginal land in the uplands will become more important for raising livestock. Already it has been shown that non-domestic species such as the Red Deer in Scotland and the Eland in Africa may produce meat more efficiently than cattle or sheep on poor grazing, and some scientists think we should make more effort to find new species to domesticate. Unfortunately deer and antelope have proved difficult to manage in large numbers, so their potential is limited.

Some of the old breeds may be as good at making meat from poor herbage, and we may benefit from their heritage of domestication. I have kept Soay sheep, the breed still found on the islands of St Kilda off the west coast of Scotland and apparently little changed since they were herded by Neolithic man, on pasture on which ordinary domestic breeds would have starved. The animals flourished and bred. Other breeds of sheep and cattle, unsuitable for the intensive farm, might do equally well.

correspondence

The toxicity of plutonium

SIR,—In your editorial "Take your time Mr Benn" published on September 30 you discuss the Report of the Royal Commission on Environmental Pollution. You state that "the report first reviews what is known in radiobiology with particular attention to plutonium", and that "independent consultants were brought in. . . . The consultants' reports have not been published." Two of the signatories of this letter (F.W.S. and J.V.) are acknowledged by name in paragraph 537 of the report. We wish to make it clear that the two other signatories (R.D. and P.L.), who are members of the Commission, take full responsibility for Chapter II on Radioactivity and Radiobiology in the report. This was not seen by the consultants at any time prior to publication. In fact they disagree with several of the statements made in this chapter.

In paragraph 66, for instance, the statement that "plutonium isotopes are retained in the body once they gain admittance" is certainly misleading. Excretion does occur though it is not rapid, while no mention is made in paragraph 55 of the salient fact that the energy deposited in the tissues by the strontium 90 decay process is about 200 times greater than that deposited by tritium.

Further, the greater part of the evidence given to the Commission by the consultants has already been published in their joint paper in *Nature* (February 19, 1976) entitled "Hazards of plutonium with special reference to the skeleton", and in a paper by Janet Vaughan entitled "Plutonium—a possible leukaemic risk", in W. S. S. Jee (Ed.), *Health Effects of Plutonium and Radium*, 691–705, (J. W. Press, Salt Lake City, Utah; 1976). In fact references to these two papers were made by the Commission in Chapter II and in their bibliography.

In their written evidence to the Commission the consultants stated "We are in agreement with the conclusions arrived at by the Medical Research Council¹ and the National Radiological Protection Board² on the risk to the lung and on the question of the hot particle." Reference to both these matters was made in their paper published in February in *Nature*, and in a paper published by Mayneord and Clarke³ on which their discussion was

partly based and which was also published in the same issue of *Nature* under the title "Quantitative assessment of carcinogenic risks associated with hot particles". The substance of the consultants' comments on papers submitted by the National Radiological Protection Board on the numbers of deaths from leukaemia at the Windscale reprocessing plant are contained in paragraphs 74 and 75 of the Report.

Yours faithfully,

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¹ *The Toxicity of Plutonium*, Medical Research Council, HMSO (1975).

² Dolphin, G. W., Smith, H., Popplewell, D. S., Stather, J. W., Adams, N., Spoor, N. L., Brightwell, J., and Bulman, R. A., *Radiological Problems in the Protection of Persons exposed to Plutonium*, NRPB R.29 (1974).

³ Mayneord, W. V., and Clarke, R. H., *Nature*, **259**, 535 (1976).

Genetic manipulation

SIR,—The contribution of John Locke, the Director of the Health and Safety Executive, to the debate on genetic manipulation raises a new and disturbing issue. The Director claims that "the techniques described as 'genetic engineering' should be permitted where they offer prospects of social benefit" subject to safety precautions. So we learn that the safety of experiments is not the only criterion on which they are to be judged; officials are also to pass judgment on the social benefit of genetic experiments.

The ethical problems were not even discussed in the Williams Report, which confined itself solely to safety considerations in genetic manipulation. Clearly the Health and Safety people have no mandate to assess the social benefits of work. Besides the safety aspects of work in genetics, ethical problems could arise and these need very full debate before they have any in-

fluence on executive action.

Yours faithfully,

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Human anatomy

SIR,—The interest in descriptive asymmetry shown in *Nature's* pages indicates that the classic German treatise on the subject¹, reprinted in 1970, deserves recognition by the English-speaking world. Asymmetry of human limbs, and much else, has a large literature but lacks a recent review. There is even a more recent paper² devoted to a positive correlation between handedness and scrotal asymmetry. There are also by now several dozen analytical treatments^{3–8}; fluctuating asymmetry (but not other kinds) is a measure of the imprecision of development.

Yours faithfully,

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¹ Ludwig, W., *Das Rechts-Links Problem im Tierreich und beim Menschen* (Berlin, Springer; 1932).

² Chang, K. S. F., Hsu, F. K., Chan, S. T., and Chan, U. B., *J. Anat.*, **94**, 543–548; 1960.

³ Van Valen, L., *Evolution*, **16**, 125–142; 1960.

⁴ Whitten, M., *Genetics*, **54**, 465–483; 1966.

⁵ Bailit, H. L., Workman, P. L., Niswander, J. D., and Maclean, C. J., *Human Biol.*, **42**, 626–638; 1970.

⁶ Jackson, J. F., *Syst. Zool.*, **22**, 166–170; 1973.

⁷ Salzano, F. M., and Benevides, F. R., *Amer. J. Phys. Anth.*, **40**, 325–328; 1974.

⁸ Siegel, M. I., and Doyle, W. J., *J. Exper. Zool.*, **191**, 211–214; 1975.

Careers in science

SIR,—There has been a great deal of discussion recently about possible short term cutbacks in 'big science' projects. Nowhere have the effects on the careers of scores of research students been discussed. Simultaneously there are complaints about the lack of prospective students for university science courses. Is it not possible that these two sets of circumstances are directly related?

(Name and address supplied)

To be or not to be

SIR,—Isn't isn't is'nt! (October 7, page i).

Yours faithfully,

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news and views

Coexistence with insect pests

from Robert M. May

INSECTS have shared man's crops, and transmitted his diseases, from the earliest times. On a tomb erected towards the end of the Old Kingdom in Ancient Egypt (about 2,300 BC) locusts are shown eating cereals, and the Book of Joel (1, verse 4) records a remarkable sequence of entomological misfortunes wherein "that which the palmerworm hath left hath the locust eaten; and that which the locust hath left hath the cankerworm eaten; and that which the cankerworm hath left hath the caterpillar eaten". Today at least one person in six is suffering from some form of insect-borne disease (primarily 300 million from filariasis and 200 million from malaria: see *Nature*, **262**, 85; 1976), and world crop losses are estimated around 10–15%.

Agricultural societies have evolved a variety of empirical practices aimed at reducing the depredations of crop pests. In the absence of detailed understanding of the pest's population dynamics, it is hard to be sure whether any one such practice represents the optimal control strategy, or the best among similar strategies (but inferior to some qualitatively different strategy), or simply ignorance hardened into habit. Chiang (*Science*, **192**, 675; 1976) has reviewed a most interesting series of Chinese texts, which *inter alia* codify this sort of folk wisdom: it is advised, for example, that the top leaves and buds of cotton plants should be examined at 3-day intervals for eggs of the cotton bollworm, and chemical control initiated above a threshold of 15 eggs for 100 plants.

An analytical approach to the control of crop pests and forest defoliators has been pioneered by Watt, Holling, Huffaker and others. Here one starts with a fairly detailed model for the population dynamics, and tries to determine the relevant parameters from field and laboratory experiments: in effect one tries to decide whether 5 or 15 or 50 is the appropriate number of bollworm eggs, or whether some qualitatively different strategy may be better. A notable example of this approach centres on the froghopper, *Aeneolamia varia saccharina*, which

attacks sugar cane in Trinidad and elsewhere (Conway *et al.*, in *Study of Agricultural Systems*, edit. by G. E. Dalton; Applied Science, London, 1975; and Conway, chapter 14 in *Theoretical Ecology: Principles and Applications*, edit. by May, R. M.; Blackwell, Oxford, 1976). Starting from a basic model for the dynamical behaviour of the population, the effects of various control strategies are determined. The economic costs and benefits of these strategies are then calculated, in Trinidadian dollars. The optimum strategy turns out to depend mainly on the size of the first-generation brood of hoppers, and thence on the weather. Optimal control can nevertheless be maintained by use of dynamic programming techniques.

On a more general level, Southwood (in his opening address to the 15th International Congress on Entomology, in Washington, DC on August 18) and Conway (*op. cit.*) have recently used contemporary ecological notions to bring some order and pattern to the bewildering variety of dynamical behaviour of insect pests.

At one end of a continuum are the 'r pests' (*Nature*, **257**, 737; 1975), which are characteristic of unpredictable, transient or patchy habitats: examples are desert locusts or fruit flies. Their strategy is one of boom and bust, and r pests will virtually always achieve pest level if enough of them invade the crops and there is adequate time before harvest. At the other extreme, 'K pests' occur in stable habitats. Their populations are typically steady and relatively low, and they do not have a serious impact on their natural food. K pests achieve pest status when their low level of harvesting is nonetheless unacceptable to man (as is codlin infestation in a fraction of the apple crop, or ectoparasite holes in a commercial hide), or when the introduction of plants or animals to new areas disturbs natural balances (the mirids that, even at low population levels, are destructive of the cocoa trees introduced into West Africa and the Far East inflict relatively little damage on their natural host plants;

trypanosomiasis, carried by tsetse flies, is rarely lethal to the indigenous African fauna, but is a problem for man and his domestic animals). In between these extremes are the majority of deciduous forest defoliators, fruit insects and some vegetable pests. These 'intermediate pests' are for much of the time held at a lower equilibrium point by natural enemies, but they occasionally escape this natural control and reach outbreak levels. Those near the r end of the spectrum, such as aphids, escape most frequently. Intermediate pests may become r pests when they are introduced to new areas where their natural enemies are absent: examples of such introductions, where the organisms are not significant nuisances in their countries of origin, are the gypsy moth, *Lymantria dispar*, to North America, and the giant African snail, *Achatina fulica*, to Pacific islands. This continuum of r, intermediate and K pests is elaborated and fleshed out with numerical examples in Southwood and Comins (*J. Anim. Ecol.*, **45**, 949; 1976).

Southwood observed in his opening address that "this knowledge of the underlying population dynamics of different pests gives useful indications of the appropriate pest control methods". The term control is here used in a precise and sensible way to mean reducing the pest impact to a level where the marginal cost of further measures would exceed the marginal revenue to be gained thereby.

In Southwood's words, "r pests are always fluctuating, and therefore any appeal to the stability of natural ecosystems is spurious and foredoomed to failure". Cultural control techniques will depend on reducing the chances of pest invasion. Paradoxically, and contrary to tenets laid down in simple ecology tracts, one way of doing this is to increase the scale of a monoculture, thus increasing the area-to-perimeter ratio and heightening the degree of isolation (Way, in *Biology in Pest and Disease Control*, edit. by Price-Jones and Solomon; Blackwell, Oxford, 1974). However, in these "inherently booming populations, in-

secticides will remain the most powerful technique: their rational use will demand the development of better methods of forecasting and assessing pest outbreaks".

Intermediate pests should be kept below their release point, so that the population is stabilised by natural enemies. Southwood emphasises that these are the pests against which biological control, or integrated control with a significant natural enemy component, must be the dominant strategy. When pests of this intermediate kind are present in an ecosystem, the mindless "prophylactic" application of insecticides is likely to eliminate the lower equilibrium point controlled by natural enemies, thus leading to outbreaks of "secondary" or "upset" pests.

K pests will be the most susceptible to eradication, or at least to being kept at low population levels. They often have complex reproductive tactics and these, together with the relatively low rate of recruitment of the adult stage, make them vulnerable to techniques such as the release of sterile males (as

in Knippling's work on the screw-worm fly, *Cochliomyia hominivorax*) or the use of pheromones.

These insights imply that insecticides are likely to remain a vital tool in engineering our coexistence with insects. On the other hand, the past 30 years have seen a faster-than-exponential rise in the proportion of pests resistant to insecticides: over 200 species of significant pests are now resistant to one or more chemical compounds. Eradication programmes based on the heavy, widespread and prolonged use of insecticides are not only biologically naive and usually futile, but they also squander a valuable resource—the life of the insecticide. The obvious conclusion, emphasised by Southwood and by the US National Academy of Sciences study committee on pest control (*Science*, **191**, 836; 1976), is that we must in the future be more selective in our use of insecticides, and more efficient in their application, than we have been in the past. In this endeavour, analytical understanding of the population dynamics is a crucial ingredient. □

Giordano Bruno, the Moon's latest large crater

from David W. Hughes

To the observer with a telescope the Moon's surface seems unchangeable and over three centuries of study has revealed no well documented alteration. This is hardly surprising considering that, in the absence of erosion, the cratered surface has taken more than four thousand million years to get to its present state. Also recent estimates of the present day cratering rate (Shoemaker, *NASA Tech. Rep.* No. 32-700) indicate that a 1-km crater (which is just on the seeing limit for Earth-based telescopes) will only be formed on average once every 4×10^7 yr. A crater of 20 km or larger is produced every 3×10^{11} yr, which in view of the fact that there are many such craters on the Moon is ample indication of how the large body influx and thus the cratering rate has decreased with time.

The above points make the paper by Jack Hartung (State University of New York, Stony Brook) in the recent edition of *Meteoritics* (**11**, 187; 1976) all the more fascinating. Hartung retells the report of five men who happened to be looking at the new Moon early in the evening of July 18, 1178. The Moon was then 1.5 d past new and would have been seen as a thin waxing crescent setting near the western horizon in the evening twilight glow.

"... suddenly the upper horn split in two. From the midpoint of this division a flaming torch sprang up, spewing out over a considerable distance fire, hot coals and sparks. Meanwhile the body of the Moon which was below writhed, as it were in anxiety ... and throbbed like a wounded snake. Afterwards it resumed its proper state. This phenomenon was repeated a dozen times or more, the flames assuming various twisting shapes at random and then returning to normal. Then after these transformations the Moon from horn to horn, that is along its whole length took on a blackish appearance".

This report was given on oath to Gervase of Canterbury and incorporated in his mediaeval chronicles (edit. by Stubbs, W., *The Historical Works of Gervase of Canterbury*, **1**, Her Majesty's Stationery Office, London, 1879).

It is highly unlikely that some Earth-based event caused this phenomenon although an unusual cloud layer or turbulence in our atmosphere or the entry of a meteoroid along the line of sight to the Moon could have been responsible. The report, however, repeatedly refers to the Moon and does not mention clouds or sky specifically.

Hartung suggests that the description

is consistent with the occurrence of a large impact on the lunar surface. "The upper horn split in two"—part of the sunlit crescent became obscured by the cloud of ejecta produced by the impact. "A flaming torch sprang up, spewing out fire, hot coals and sparks"—a vivid description of the hot dust, molten rocks and incandescent gases ejected from the impact point. "The moon writhed and throbbed like a wounded snake"—the Moon seen through the highly turbulent temporary atmosphere produced by the ejecta. "This phenomenon was repeated a dozen times or more"—the production of secondary impact craters by large pieces of ejecta that had insufficient energy to escape from the gravitational attraction of the Moon and fell back onto its surface. "The Moon then took on a blackish appearance"—the entire Moon's now having a temporary dusty atmosphere which blocked a significant amount of the light reflected from its surface.

Does such a newly formed crater exist on the Moon? The event occurred near the midpoint of the upper horn of the new Moon so the area around latitude 45° north, longitude 90° east is a good place to start looking. It was also easily visible to the naked eye indicating that the ejecta cloud was more than 100 km across. The crater should therefore be more than 10 km in size. Also a recent crater should have a bright ray system (rays being constant-width fingers of light coloured ejecta which radiate from the area of the central crater along the arcs of lunar great circles).

Searching the area between latitude 30° and 60° N and longitude 75° and 105° E using Lunar Orbiter and Apollo mission photographs Hartung soon found a likely candidate, the crater Giordano Bruno (latitude 30° N, longitude 103° E). This is 20 km in diameter and is surrounded by very prominent bright rays which extend for hundreds of km (see NASA photograph AS8-12-2209). In fact Giordano Bruno has the largest ray length-to-crater diameter ratio of all the lunar craters. The crater rim is also very sharp and has not yet been rounded off by the "gardening" process as have the older craters (Fig. 1).

It is interesting to think how this theory can be verified. Assuming that the observation was made near Canterbury, Hartung finds that on June 18, 1178, the Sun set at about 20.15, the Moon setting about 45 min later, so the event must have occurred between 20.15 and 21.00 GMT. Also making the assumption that the Sun had to have set for the event to be visible, limits the points on the Earth from which it could be seen to a band stretching from the region between

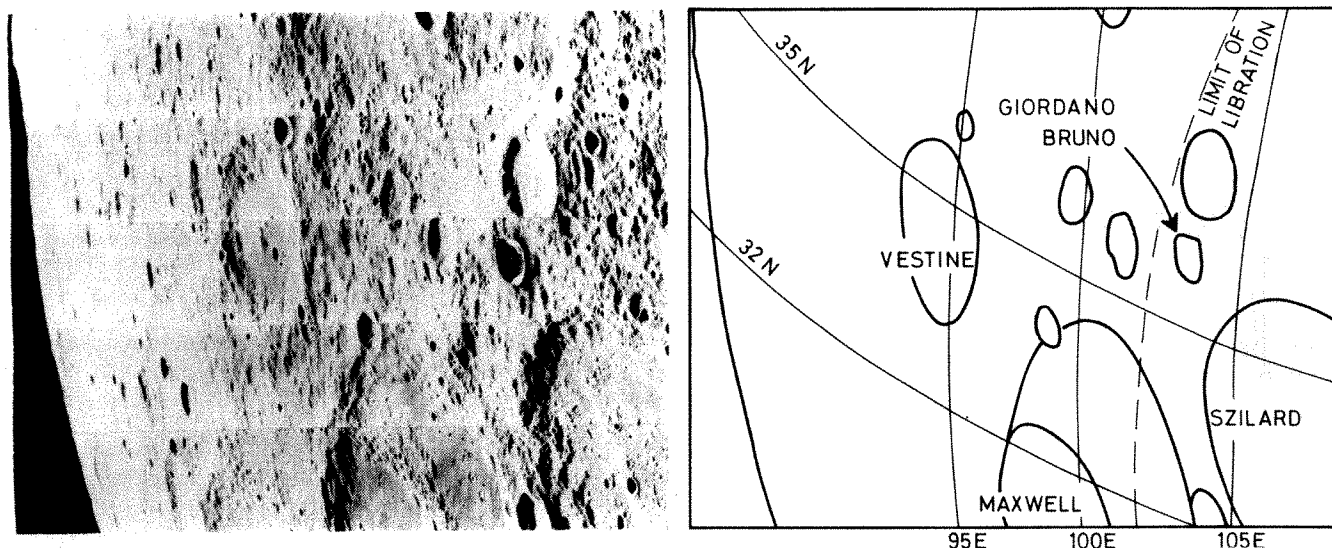


Fig. 1 A Lunar Orbiter photograph of the Moon's latest large crater, Giordano Bruno. The impact producing this crater was observed from near Canterbury, England at around 20.40 Greenwich Mean Time on June 18, AD 1178 (courtesy of NASA).

Oslo, Norway and Stockholm, Sweden, through southern England and the north-west corners of France and Spain, west of the Canary Islands and along the south-east coast of Brazil. Mediaeval records from these countries should be searched to see if the event is mentioned.

The crater itself might still have the signature of its recent formation. Microwave sensors on board a lunar polar orbiter may still be able to detect a higher temperature inside the crater, attributable to the heat generated during the original impact. Also rocks ejected from the crater will have been exposed to space for only 800 yr and will have a distinctive population of micrometeorite craters and cosmic ray particle tracks. The return of such samples to Earth will not only prove the craters to be of recent origin but will also be a valuable clue to other long time-scale Solar System processes such as the implantation rate of solar wind particles and cosmic ray nuclear reaction rates.

All in all this crater identification is most exciting. Even taking Baldwin's (*Icarus*, 14, 36; 1971) Earth cratering rate as applicable to the Moon the chances of anyone observing the formation of a large lunar crater on the Earth-facing side of the Moon during the millennia of recorded history (say 3,000 yr) is 1 in 2,000. Because observations have not been made continuously, may not have been recorded or the records might have been lost, the probability becomes extremely small and the Canterbury record even more fortuitous. Discounting the dubious and controversial changes claimed to have occurred to the crater Linné in Mare Serenitatis (Fauth, *Astr. Rundschau*, 3, 172; 1901) Giordano Bruno is unique, being the only large lunar

crater to be formed in recent history and as such it becomes a prime lunar target for future space exploration.

Drugs and body temperature

from a Correspondent

The Third Symposium of The Pharmacology of Thermoregulation, was held at Banff, Canada, on September 14-17, 1976.

"AND now for something completely different!" This sentiment imbued many of the presentations at the conference. 'Thermopharmacology', a term coined in 1969, is devoted to the relation between the action of a drug and an animal's body temperature in all its complexity, and it is difficult to know where pharmacology ends and physiology begins in this subdiscipline. In fact, much of this conference did deal with functional rather than pharmacological questions—particularly those centred on the body temperature control mechanism located in the brain. Overall, there were several exciting highlights.

"What is the purpose of a fever and what is its value to the survival of the organism?" In asking this question, M. Kluger (University of Michigan Medical School) laid a potential bombshell in the physician's lap. In an elegant experiment, he demonstrated that aspirin is not beneficial after all

when given to a rabbit, desert iguana or other animal during a fever caused by bacteria. In fact, if given a dose of aspirin that prevents fever from developing, a large proportion of the animals die. Following on, J. Covert and W. Reynolds (Pennsylvania State University) reported independently that goldfish, small-mouthed bass and other fish manifest a "behavioural fever". After infection with bacteria, the poikilotherm, living in an aquarium heated along a gradient, swims to warmer water. If kept in cooler water, the bacteria-laden fish dies. If an aspirin-like compound is dissolved in the aquarium water, an afflicted fish will likewise succumb, since it fails to swim to warmer water after the antipyretic drug is taken in, presumably through the gill network. Even though a fish does not regulate its body temperature in the same way as a mammal, the nervous system of this evolutionarily more primitive animal is nevertheless affected by the pathogen. So aspirin usage may have been dealt yet another blow.

A new "activator substance" released by white blood cells was described by G. Gander (Medical College of Virginia). This protein possesses the property of triggering other types of inflammatory cells to release a pyrogen. The "activator" could account for the fever seen in a patient who is stricken with a heart attack yet exhibits no signs of an infectious agent. Observations by E. Atkins (Yale University) implicate a lymphokine released from blood lymphocytes that causes other blood cells (monocytes) to produce pyrogen. A fascinating question now is whether the lymphokine and the "activator" are one and the same.

Along these lines, a new assay for

identifying "pyrogenicity" (the fever-producing capacity of a substance) was evaluated by G. Meers (Calgary Medical School). If *Limulus* (horseshoe crab) blood is mixed in solution with quite minute amounts of bacteria, the crab's blood thickens. Conceivably, in a patient who has contracted an infectious disease, bacterial counts in blood could be quantitated; alternatively, the crab blood assay could be used for the relatively rapid screening of drug solutions intended for administration to the human patient.

In several quarters, pockets of distinct but congenial controversy arose. What, for example, is the role of the metabolic substrate cyclic AMP in the temperature control mechanism? A. Milton (University of Aberdeen) reported that cyclic AMP and other cyclic nucleotides produce hypothermia on their injection into the hypothalamus of the cat. But exposure of the animal to a hot or cold environment fails to alter the central level of cyclic AMP. A contrary result was then presented by C. Rosendorff (University of Witwatersrand, Johannesburg) who contended that, in the rabbit, cyclic AMP evokes hyperthermia and indicated that cyclic AMP could be an intermediary for producing fever. An easy resolution would be to fall back upon "species difference" as an explanation. Recent evidence continues to accumulate which implies that different mammalian species have similar neural and humoral mechanisms for heat gain and heat loss. In fact, it was revealed that in the rabbit 5-hydroxytryptamine (5-HT) causes hyperthermia, and noradrenaline, hypothermia, which are precisely the same responses seen in the cat, rat, monkey and other species. Expertise in technique, or lack thereof, rather than nature's capriciousness may ultimately explain the so-called "species difference". In any event, the "second messenger" question concerning cyclic AMP has yet to be resolved.

An even more intriguing controversy is the role of the prostaglandins (PG) in the brain and the part they play in fever induced by bacteria. Ostensibly, bacteria cause an increased synthesis and/or release of a PG in the brain which subsequently produces a long-lasting hyperthermia. R. Hellon (National Institute for Medical Research, London) shook the theory by announcing that an antagonist of PGE₂ (a compound coded as SC 19-220) blocks a fever evoked by this prostaglandin. Thus, if the pyrogen-PGE theory were correct, this antagonist should likewise prevent bacterial fever. But it does not. This suggests an alternative mediator. R. Myers (Purdue University) troubled

the waters further by showing that a low dose of aspirin perfused in the cat's hypothalamus, although blocking PGE activity in this structure, fails to prevent a bacterial fever from developing. However, 5-HT activity in the hypothalamus increases simultaneously with the rise in body temperature after infection with bacteria. Thus, 5-HT may be the mysterious intermediary in the hypothalamus after all. C. Rosendorff (Johannesburg) informally countered that a part of the PGE hypothesis may still be valid, since a precursor of PGE or a factor such as an endoperoxide (thromboxane) in an alternative pathway could be causal intermediaries. Feldberg ended by describing how PG-induced and other fevers can now be examined in the anaesthetised animal, given the appropriate anaesthetic. The brain mechanism responsible for fever and its development is still tantalisingly elusive.

Ionic mechanisms in the hypothalamus also exert an apparent influence in the final common neuronal pathway underlying temperature control. D. Jones (Calgary Medical School) showed that the hypothermia induced by calcium ion perfusion of the cat's hypothalamus is antagonised by adding glucose to the perfusate. This observation suggests that a local energy substrate can modify the ionic activity of the posterior hypothalamus. The endogenous activity of Ca²⁺ ions, traced in the same region of the cat's brain by R. Myers (Purdue), changes in an opposite direction when the animal is warm or cold as well as when its hypothalamic thermoreceptors are warmed or cooled. In spite of this, the issue of whether or not an ionic mechanism does underlie the typical set-point temperature of 37 °C persists.

Ingenious techniques and their use were also emphasised. For example, the behavioural assessment of drugs that affect temperature regulation is now a popular procedure for deciding whether or not an animal will try to avoid being cold or warm after a drug is administered (E. Adair, Yale; E. Satinoff, University of Illinois). Although the importance of diurnal rhythms was also forcefully stressed by F. Halberg (University of Minnesota), one wonders whether the use of a control animal at the same time of day or night as an experimental subject would really make it possible to interpret let us say, a thermal effect of a given drug. Finally, the radioactive microsphere techniques developed recently were described by R. Hales (CSIRO, Sydney). These techniques are useful in obtaining a comprehensive picture of the effects of thermal stimulation on the regional distribution of cardiac

output and blood flow.

With the advent of the Feldberg-Myers monoamine theory of thermoregulation more than 10 years ago, pharmacologists have often concluded that a drug affecting body temperature does so by altering the balance between 5-HT and noradrenaline activity in the animal's hypothalamus—the essence of this theory. At the conference, it was evident that endogenous substances other than these amines will have to receive equal time at the research bench. Thyroid releasing hormone (TRH), prostaglandins, calcium, dopamine and other substances could be important factors mediating the brain's response to a drug given systemically. Of course, the relevance of these substances to synaptic events with regard to the hitherto prominent role of biogenic amines is still uncertain. □

Control systems in higher plants

from John A. Milburn

A symposium entitled Integration of Activity in the Higher Plant was held in Durham, under the auspices of the Society for Experimental Biology on September 4-9, 1976. The proceedings will be published.

BUMBLE-BEE flight physiology and rocket guidance systems are unexpected topics to meet in a plant physiology symposium. Nevertheless such examples served to illustrate the theme of the meeting, although those expecting papers on integration may have been disappointed; most emphasis was on control systems, probably because information on integration is so sparse.

The regulation of the heating up of the *Arum* spadix has been studied by T. ap Rees (University of Cambridge). Biochemically, the *Arum* spadix is a curiosity: in nature it heats up to more than 10 °C above ambient temperature, releasing a scent that attracts *Psychoda* midges which pollinate the flower. Heating is achieved by intense respiration comparable to that in the most physiologically active tissues such as the flight muscle of bats, birds and insects. This exaggerated respiratory system is used by ap Rees to study the inter-relation between glycolysis and the pentose phosphate pathways. A search for a mechanism in plants like the 'free-wheeling' or 'futile' cycles, responsible for warming up bumble-bee flight muscles in cold weather, has not been successful in *Arum*.

Why do stored seeds lose their viability? D. J. Osborne and colleagues (ARC Unit of Developmental Botany, Cambridge) attribute the loss of capacity of rye grains to germinate, to the progressive proliferation of embryonic lesions. With time, lesions in embryonic tissues increase beyond the point at which germination is possible, producing a state in which rye is 'deader than dead'. Lesion formation seems to result in failure to reactivate nucleic acid and protein synthesis sufficiently rapidly. Curiously, if rye grains are subjected to a preliminary brief wetting and drying cycle before sowing, reactivation is improved and viability and embryo vigour are enhanced over controls.

One would think that the flow of the transpiration stream through plant organs would have few mysteries by now since the water flow is generally believed to be regulated predominantly by the physical properties of the cell walls. On this basis the hydraulic conductance should be constant irrespective of the water potential gradient. J. S. Boyer's (University of Illinois, Urbana), results, like those from other laboratories, show that conductance increases dramatically as the water pressure gradient increases, which is not expected of a simple physical system. There was no obvious agreement as to the explanation of this intriguing phenomenon.

Generations of students have deduced cell turgor pressures by immersing pieces of tissue in osmotic solutions on the assumption that the internal solute concentration remains constant. This assumption is now strongly challenged by U. Zimmermann (Kernforschungsanlage, Jülich) who has measured the turgor pressure of single cells using a microprobe and pressure transducer. It is clear that cells respond directly to turgor pressure because, in addition to the anticipated water transport, the injection of inert fluid into cells produces immediate changes in the fluxes of solutes, especially potassium. Similar methods have been used to determine the elastic modulus of *Valonia* and *Mesembryanthemum* cells. How pressure modifies ion fluxes is a mystery. The geometry of the plasma membrane is apparently disturbed but the full explanation will be more complex. Although cell membranes can be compressed mechanically the technique is tedious but Zimmermann has introduced a revolutionary electrical method which charges membranes in the same way as the dielectric of a capacitor. Many new advances can be expected of this technique which has already been used to punch temporary holes in erythrocyte mem-

branes, causing them to absorb drugs. These erythrocytes can be reinjected and are being tested for greater control in the treatment of cancer.

The regulation of ions and solutes in cells and plant organs was outlined by J. A. Raven (University of Dundee), then M. G. Pitman and W. J. Cram (University of Sydney). Nowadays the driving system supplying energy for ion transport is widely believed to depend on proton pumping in accordance with Mitchell's chemiosmotic hypothesis. Proton pumping, if linked directly or indirectly to other ionic fluxes can explain many physiological responses, such as stomatal regulation, auxin transport and cell expansion, in addition to ion uptake by plant roots. The lipid bilayer of cell membranes has a high electrical and hydraulic resistance and to drive solutes at the rates commonly observed the membranes must contain ion pumps. The regulatory control of ion pumping is seen in the response to specific ions and also to growth regulating substances. In whole plants the uptake of cations and anions tends to be balanced and proportional to the amount of shoot growth but how the plant maintains the correct balance is not known. Is carboxylic acid production in leaves sufficient to balance cation uptake, for example, and is the extrusion of hydrogen ions from roots sufficient to explain cation uptake?

The orientation of plant organs requires sensing and response systems analogous to those in guided rockets, according to M. B. Wilkins (University of Glasgow) explaining recent progress in research on phototropic and geotropic responses. A strong plea was made for greater precision in describing and interpreting "hormone" experiments. Bioassays are too ambiguous for critical work and must be supplanted by physicochemical techniques such as mass spectrometry, used recently to confirm that the coleoptile geo and phototropic responses are in fact caused by indole acetic acid (IAA). Control systems are complex, varying between different organs and tissues and involving an interplay of growth promoters and inhibitors which operate in minute dosages. In *Zea* roots, growth inhibitors are undoubtedly involved in geotropic curvature, current evidence favouring a role for abscisic acid (ABA) rather than IAA. Doubt was cast on the popular view that sedimenting starch grains initiate the geotropic response.

Two theoretical papers by I. R. Cowan (Australian National University) and J. H. M. Thornley (National Vegetable Research Station, Littlehampton) gave refreshingly clear ex-

positions of mathematical model building. Their models sought to explain how variations in stomatal aperture might optimise productivity in arid zones and how crop yield might be predicted from measurements. Characteristically, those who felt such models provided a useful insight into their problems reacted favourably, but others felt the degree of abstraction too far removed from current technology to be very useful.

Other topics covered included aspects of carbon metabolism and translocation, cell and apical growth, and rhythms and hormonal integration.

Although the papers varied considerably in the viewpoint which was taken this symposium provided something for everyone. The organisers are to be congratulated for providing an invaluable platform which was educational and extremely enjoyable for all concerned, and the published text is awaited eagerly. □

Miraculous peat?

from F. M. Slater

The 5th International Peat Congress was held in Poznan on September 21-25, 1976.

ONE interesting aspect of the Congress was the claim made for peat and peat preparations as a source of antiviral compounds, and of their use in the treatment of cancers, osteoarthritis and conditions arising from bronchial asthma. W. Adamek of Wroclaw, a medical practitioner, described 13 case histories of people with cancers of the skin, breast or alimentary canal who were treated using a peat extract developed by S. Tolpa (Academy of Agriculture, Wroclaw). Regression of the tumours was evident in ten of the patients when the preparation was administered orally (with intramuscular supplements in the case of breast cancer, rectal supplements for cancer of the lower bowel and poultices in the cases of skin cancer), and three reacted adversely to the treatments.

H. Wrobel and C. Jasiak (Janusz Korczak Hospital, Wroclaw) described how a peat preparation, named only as TK-, was beneficial in the treatment of hyperacidity which usually occurs in patients with bronchial asthma. Fifty patients were treated with the preparation which was administered orally before meals in doses of 30 g three times per day for 3 months. This treatment generally resulted in the complete disappearance or decrease of abdominal discomfort, disappearance

of distention, dull pains and unpleasant taste in mouth. Although the treatment obviously had a beneficial effect the authors admit that the mechanism of the therapeutic action remains unknown.

In much of Europe, unlike Britain, spas, peat baths and indeed the general study of balneology are still very much a feature of the medical scene, and K. Weber and G. Plötner of the German Democratic Republic presented the results of various peat bath therapies in the treatment of rheumatoid arthritis. They concluded that whatever peat consistency they used, all their patients benefited by a reduction in their erythrocyte sedimentation rate.

Although the successful treatment of foot-and mouth disease with peat litter containing humic acids has been demonstrated in the past, little recent work has been done on the antiviral properties of the humic acids of peat. R. Klöcking and M. Sprössig together with T. Klaus-Dieter (Medical Academy, Erfurt), however, presented a paper which demonstrated that ammonium humate possesses antiviral activity against herpes simplex virus types 1 and 2 when used in a concentration between 1 and $10 \mu\text{g ml}^{-1}$. The most humate-sensitive phase of virus multiplication seems to be the adsorption of viruses to the host cells. This report was supplemented by other work from the same institution by J. Witthauer, R. Klöcking, B. Helbig and P. Drabke who reported that the molecular weight of eluted humate was in the range 10^3 – 1.2×10^4 with a peak for ammonium humate at 3.3×10^3 . They also described a sensitive method for the detection of humic acids by means of polyacrylamide gel electrophoresis and staining with 0.2% alcian blue.

Although the congress went on to deal with peatland technology and conservation, it was the uses of peat for environmental protection which caught the imagination of delegates. Peat has been demonstrated to be an effective environmental filter for many substances. B. Illarionovich, G. Alexandrovna and C. Romanovna (Academy of Sciences, Minsk), described how peat's initial and natural affinity for elements such as Cu, Co, Zn, Ni, Ag and Ti can be increased 1.5 to 3-fold by previous oxidation of the peat with nitrogen tetroxide. Z. Rozmej and A. Kwiatkowski (Technical University, Gdansk) demonstrated the importance of brown coal in addition to peat as an ion exchange for metals including Al, Cr and perhaps most importantly U.

D. Asplund, E. Ekman and R. Thun (Technical Research Centre, Espoo) found peat to be effective in the puri-

fication of oily water. Untreated peat had an oil-binding capacity of 1–2 kg oil per kg peat, rising to 3.4 kg oil per kg peat when heat-treated peat was used.

In areas of high environmental value, such places as small camp sites and lay-bys may require a high purity of filtered wastewater such that very low nitrogen and phosphorous levels are present in the effluent. This demand combined with the usually low flow rates in such situations makes the wastewater filtration system described by J. L. Brown and R. S. Farnham (University of Minnesota, St Paul) the ideal answer. The basic design consists of 15 cm of coarse gravel covered by 50 cm of medium to fine sand topped by 30 cm of peat in which a crop cover is grown. The design can be modified by the addition of CaCO_3 to the peat to induce calcium phosphate precipitation or by an additional submerged peat layer to aid denitrification. A circular filter bed of this type 30 m in diameter would be adequate to treat the waste from 80 persons at the rate of 30 cm per week. Not only are effluent nitrogen and phosphorus very low but also, after three summers of operation, removal of total coliform bacteria remains nearly complete. \square

Itinerant-electron magnetism

from E. P. Wohlfarth

A conference on Itinerant-Electron Magnetism was held in Oxford on September 13–15, 1976. It was sponsored by the Magnetism Section of the European Physical Society and the Institute of Physics. The Proceedings will be published next Spring in the *Europhysics Journal Physica B*.

ALTHOUGH the birth of this subject took place some 40 years ago it was not until this September that a full scale conference was devoted to it. The meeting was concerned with the magnetic and related properties of metallic materials as influenced by their energy band structure, the interactions between the itinerant, that is, non-localised electrons, and the proper statistical mechanics determining thermal effects. This combination of influences leads inevitably to such a complex of physical problems that no progress is possible without the most judicious interplay between theory and experiment. Even 40 years turned out to be by no means too long a period to wait for an adequate summary of the status of this subject.

Several conflicting philosophies were

clearly revealed and their full resolution is just as clearly not yet at hand. One of these is concerned with the importance of spin fluctuation effects which T. Moriya (University of Tokyo) described. These effects give rise to serious modifications to the original Stoner model of itinerant magnetism which combines the molecular field approximation with Fermi statistics. D. M. Edwards (Imperial College, London) also discussed the importance of electron correlation effects in transition metals, and J. Hertz (University of Chicago) used an advanced many-body approach to discuss "sloppy" spin waves above the Curie temperature. Expressions of continuing support for the Stoner model in iron, nickel and cobalt were given by M. Shimizu (University of Nagoya). E. P. Wohlfarth (Imperial College, London) felt the case for nickel not proven but that another class of alloys, weak itinerant ferromagnets whose Curie temperature is low, continues to exhibit sufficient evidence for this model, especially where magnetoelastic effects are concerned. New measurements on this aspect were provided by G. Hilscher (Technical University of Vienna) and K. H. J. Buschow (Philips Research Laboratories, Eindhoven). On the other hand S. Ogawa (Electrotechnical Laboratory, Tokyo) and Y. Masuda (University of Nagoya) produced experimental evidence for the importance of spin fluctuations in describing dynamic properties of weak itinerant ferromagnets. M. Brodsky (Argonne National Laboratory) described evidence for related many-body effects in the specific heat of some actinide compounds.

A popular field of research in the subject concerns the spin polarisation of electrons emitted from ferromagnetic metals and alloys. R. Meservey (National Magnet Laboratory, MIT) described measurements showing that the polarisation of electrons tunnelling from alloys follows the static magnetisation closely. W. Eib (ETH, Zürich) showed new photo-emission data for single crystal nickel specimens which showed an expected low energy sign reversal. A feeling thus arose, perhaps somewhat dangerously, that the ground state of nickel is fully understood using a simple band approach. M. Campagna (Bell Laboratories) also gave supporting evidence.

C. G. Windsor (Harwell) and Y. Ishikawa (Tohoku University, Sendai) described old and new data obtained by the most fruitful of all experimental techniques, involving neutron scattering. Among other results such measurements give valuable information on the properties of the elementary excitations in itinerant ferromagnets, the single particle and spin wave excitations.

Both of these were claimed by P. C. Riedi (University of St. Andrews) to have been observed by nuclear magnetic resonance measurements. A. J. Freeman (Northwestern University, Evanston) reported a wealth of calculations of neutron magnetic form factors and related quantities in metals.

Other magnetic materials whose properties may be discussed on the basis of an itinerant electron model include amorphous metals and alloys which were described by G. S. Cargill (IBM, Yorktown Heights) and M. J. Zuckermann (University of Paris, Orsay). R. D. Lowde (Harwell) and W. Young (Queen Mary College, London) gave a theoretical account of antiferromagnetic metals whose properties under stress were described by R. Griessen (Free University, Amsterdam) and E. Fawcett (University of Toronto), concentrating on chromium. More involved results on such materials, involving conductivity transitions, were described by J. M. D. Coey (CNRS, Grenoble).

Whereas the Hubbard Hamiltonian, discussed in its simpler aspects by M. Cyrot (CNRS, Grenoble) is a very use-

ful tool for describing the interactions between the itinerant electrons which are fundamental to the whole range of observed phenomena, an equally basic *sine qua non* are the energy bands themselves, both in pure metals and in alloys. New energy band calculations in nickel and iron were described by J. Callaway (Louisiana State University, Baton Rouge) who was able to estimate the parameter of the Hubbard Hamiltonian. Similar results were also obtained by O. K. Andersen (Technical University, Lyngby) and O. Gunnarsson (Institute of Theoretical Physics, Gothenburg). The approaches of these three talks present a new era of accuracy in band calculations as relevant to the magnetism of metals. J. Kanamori (University of Osaka), on the other hand, summarised the results of the coherent potential approximation in alloy calculations.

In the introduction to the Conference mention was made of the giants of the subject who were unable to be present. One of these, J. C. Slater, had only recently died and the Conference remembered his pioneering work with humility. □

sence of histone, it was found that only 50% of reticulocyte NHPs bind to DNA in the absence of histone and NaCl, suggesting that some NHPs require histones for complete reassociation. While of the 50% of the NHPs which do bind in the absence of histone, the majority elute at 0.15 M NaCl, a significant fraction elutes only at lower NaCl concentrations at which the bulk of the histones do not bind to DNA. In general the NHPs which bind to free DNA have low molecular weights and do not show species specificity when compared to NHPs from Ehrlich ascites tumour chromatin.

Thus Gadski and Chae have begun to survey a range of characteristics of the individual proteins which comprise the NHPs of chromatin. Knowing the number of individual NHP species, their molecular weights, their relative concentrations and the conditions under which they will bind to DNA and/or to a DNA-histone complex will give clues as to the function of specific NHPs. Comparison of binding patterns such as presented here with those of the NHPs of other species might suggest which of the DNA-binding classes of NHPs are species specific and which thus might be likely candidates for key roles in the regulation of transcription. Obviously the type of experiment which Gadski and Chae present offers little information as to the specific

Non-histone proteins

from Carol K. Klukas

THE complex composition of chromatin and the intricate interactions of DNA with histones and non-histone proteins (NHPs) which define its structure are of great interest to many biochemists today because these interactions are centrally involved in the system of control of eukaryotic genome expression. The innate complexity of chromatin structure has necessitated division of the general problem into smaller and more approachable segments. Isolated histones, for example, have been studied at great length including the sequencing of the individual proteins and more recently the analysis of the interactions of histones with each other and with DNA to form nucleosomes. Because the number of different histones is quite small and because their sequences have been highly conserved through evolution, the analysis of histones has been a relatively straightforward and manageable task. The non-histone proteins contained in chromatin, however, include many more individual species, which are each present in much lower quantities than the histones, and which, moreover, probably differ considerably with species and with tissue. From numerous *in vitro* transcription and reconstitution experiments it seems quite clear that the function of at least one or a few of the non-histone proteins is to direct exactly which of the genes are to be

transcribed from the DNA in a particular tissue at a specific time. The elucidation of how they interact with each other and with the histones and DNA to accomplish this task will be very difficult to be sure; however work in this direction has already begun as illustrated by a recent paper in *Biochemistry* (Gadski and Chae, *Biochemistry*, **15**, 3812; 1976).

In this paper Gadski and Chae present their studies of erythrocyte and reticulocyte chromatin dissociated in 2 M NaCl-5 M urea. The resultant reconstituted chromatin was analysed on acid-urea-polyacrylamide gels to determine which histones had bound to the DNA, as well as on SDS-polyacrylamide gels to determine which of the NHPs had bound. Using this assay system Gadski and Chae have found that at least one major high molecular weight NHP and two minor smaller molecular weight proteins remain associated with DNA in the 2 M NaCl-5 M urea dissociation conditions. Various other NHPs associate with DNA before, during and after the association of histones with DNA as the NaCl concentration is dropped. This indicates that the NHPs include protein species with a wide range of binding characteristics.

In similar experiments in which NHPs and DNA bound to cellulose were allowed to reassociate in the ab-



A hundred years ago

IN the very interesting Address delivered by Sir C. Wyville Thomson, at Glasgow, on the *Challenger* expedition, while referring to the "red clay" deposit so general over the deepest parts of the Atlantic and North Pacific, the remarkable fact is mentioned that the clay contains numerous nodules of peroxide of manganese, which in some places are found in great quantity. The Address goes on to say:—"This is a phenomenon which we are as yet unable to explain, and I do not know that there is any analogous instance in any of the older formations" (*NATURE*, vol. xiv, p. 494).

A GERMAN paper describes a dreadful fight between two Polar bears, male and female, in the Cologne Zoological Gardens. After a fierce struggle the female became exhausted, and was dragged by the male into the water basin in the den, and held down till life was quite extinct. He then pulled her out and dragged the body for a considerable time round the den. From *Nature*, **15**, November 16, 57, 70; 1876.

mechanism whereby the NHPs participate in control of genome expression, however, their results do certainly suggest some generalities about NHPs; such as that at least some of the NHPs must bind to the chromatin complex in cooperation with histones. Certainly many more studies must be performed using chromatin from different tissues and species before systematic comparisons among them all can be made. Such comparisons it is hoped, will begin to give clues as to which of the NHPs are responsible for control of the specificity of transcription and how each of them accomplishes this function. □

European geophysics

from a Correspondent

AFTER conferences in Zurich (1973) and Trieste (1974) the European Geophysical Society held its third meeting at the Free University of Amsterdam on September 7–10.

THE meeting attracted participants from both eastern and western Europe and the United States including a significant proportion of graduate students and 'younger' research workers.

In an effort to provide a forum for as many geophysical interests as possible, and to give the meeting a more open character, the scientific organisation was changed this year into a divided programme of eight convened symposia (containing some invited contributions) and seven more loosely structured subject sessions arranged on the basis of submitted abstracts.

In the symposium on Reliability of Palaeomagnetism: Criteria, Methods and Error Estimation several authors emphasised the importance of multi-component magnetisations, and there were few contributions which treated the problem from a purely rock magnetic standpoint. Among the most notable of these was that by H. Soffel (University of Munich) who showed the domain structure in pyrrhotite from a Devonian diabase. Using the Bitter technique and with the help of a Super-8 movie, Soffel demonstrated lamellae-shaped domains, the absence of closure domains, pseudo-single domain effects and the phenomena of domain wall pinning and magnetic viscosity. Soffel later used the same technique to justify the use of basalts with low Curie temperatures ($<70^{\circ}\text{C}$) in palaeomagnetic studies. Domain structure studies at different temperatures showed that the

largest titanomagnetite grains had the lowest Curie temperatures. Smaller grains and some grain margins had the highest Curie temperatures and it was these which carried the magnetically hard part of the natural remanent magnetisation.

The problem of multicomponent magnetisation in basaltic rocks was considered by R. Løvlie and K. M. Storetvedt (University of Bergen) with R. L. Wilson (University of Liverpool). Løvlie, in a paper read by Storetvedt, discussed Tertiary basalts from the Faeroes and questioned the general validity of the baked contact test for identifying primary magnetisations, suggesting that the Middle and Upper Lava Series could have been completely remagnetised in the Middle–Upper Tertiary times. In the Skye lavas Storetvedt showed that the large range of inclinations is due to a composite palaeomagnetic record and made the point that the original direction of magnetisation isolated in laboratory treatments is not always the most significant.

Many of the papers on sediments in the same symposium carried a similar theme. R. Thompson (University of Edinburgh) reviewed the criteria for assessing the reliability of palaeomagnetic data derived from unconsolidated sediments. He stressed the need for careful consideration of the physical and chemical factors which might affect the intrinsic magnetic character of unconsolidated sediments and emphasised the importance of chemical remanence. Thompson later went on to establish minimum criteria for establishing geomagnetic excursions by example from two cores from southern Sweden which spanned the time of the supposed Laschamp event (13,000–10,000 BP). Thompson showed that low inclinations and anomalous declinations were not repeatable between these cores and indicated that they were due to inwashed sands and older material.

Doubts were also cast on the validity of the intensity minima of the geomagnetic field during polarity transitions. Løvlie developed a model based on redeposition experiments of deep sea sediments which is compatible with a zone of gradual consolidation in which alignment and immobilisation depend on the grain size distribution of the magnetic particles. The implication is that the intensity minima associated with polarity transitions are due to some intrinsic character of the sediment rather than a weakening of the intensity of the geomagnetic field.

A symposium on The Sources of Marine Magnetic Anomalies: Nature and Location showed that interpretations regarding the source of marine magnetic anomalies are being re-

thought rapidly. The notion that the anomalies reside in the oceanic basalts which form oceanic layer 2A would seem to be an oversimplification and a much thicker source layer (perhaps the whole crust) seems likely. C. Harrison (University of Miami) and N. Watkins (University of Rhode Island) demonstrated that holes drilled into the Atlantic oceanic basement to a depth of a few tens of metres do not reveal consistent magnetisation. In particular there is much variation in inclination and the implication is that the source of the anomaly is distributed throughout the oceanic crust. W. Lowrie (University of Zurich) came to a similar conclusion from the Deep Sea Drilling Project. Results from 55 sites have inclinations which do not agree with expected values; also NRM intensity shows large between-site scatter and Lowrie concluded that layer 2A shows much lateral as well as vertical inhomogeneity.

Interesting results obtained by French research workers in Antarctica were described in the environmental geochemistry session. C. Boutron (University of Grenoble) presented data, collected at South Pole station, on heavy metal concentrations in snow samples which covered the period 1950–1974. He showed how concentrations of lead and copper had increased by factors of 2 and 3 respectively over this period (though no systematic trend was found for cadmium). While the results were in general agreement with aerosol data, the author stressed how dependent the enrichment determinations were on the reliability of the reference values adopted for the mean crustal composition. Caution was also expressed against attributing the high measured enrichments to global pollution alone; the influence of local sources requires full investigation also.

Some of the stabilising effects of partial melting of upwelling mantle material at ocean ridges were considered by E. R. Oxburgh and E. M. Parmentier (Oxford University) in a symposium on Kinematics and Dynamics of Plate Tectonics. By describing the sequence in which mantle material becomes depleted by removal of high density garnet, they were able to show that density differences between the starting material and the final residue were at least of the same order as density differences arising from the typical temperature variations which drive convective motions in the mantle. Although not proposing an alternative to the thermal convection mechanism for plate motion, Oxburgh pointed out that compositional density differences can be expected to have an important modifying role in determining the form of mantle circulation. □

review article

Observational constraints on the masses of neutron stars

P. C. Joss* and S. A. Rappaport*

Direct information on the masses of five neutron stars is available at present. The mass estimates do not yet place any serious constraints on nuclear physics theory. However, if all five neutron stars have about the same mass, then the allowed mass range is quite narrow (~ 1.4 – $1.8M_{\odot}$) and is consistent with conventional pictures of neutron-star formation.

THE masses of neutron stars are of considerable theoretical interest, for two main reasons.

First, the demonstrated existence of a high mass neutron star would place constraints on nuclear physics, gravitation theory, or both. Nuclear physics in principle determines the equation of state of matter at extremely high densities. For a specific theory of gravity, the equation of state, in turn, governs the limiting mass of a stable neutron star. Following the epochal paper by Oppenheimer and Volkoff¹, much effort has been expended in estimating this limiting mass. When used to extrapolate the equation of state to densities well in excess of nuclear-matter densities ($\sim 2 \times 10^{14} \text{ g cm}^{-3}$), conventional theories of many-body nuclear physics, in conjunction with the theory of general relativity, predict a limiting mass in the range 1.7 – $2.7M_{\odot}$ (refs 2–4). However, a limiting mass as large as ~ 3 – $5M_{\odot}$ is still consistent with all direct experimental nuclear and high energy physics data^{2,5,6}. It has been shown^{7,8} that differential rotation cannot increase the limiting mass by more than $\sim 30\%$. However, if one modifies the theory of gravity, a stable neutron-star model of much larger mass can be constructed^{8,9}.

Second, in the most widely accepted picture of stellar evolution^{10,11}, a highly evolved massive star develops a degenerate core that grows by accretion of mass from the surrounding stellar envelope. As the mass of the core approaches the Chandrasekhar limiting mass ($\sim 1.4M_{\odot}$) appropriate to its composition, a thermonuclear runaway and/or dynamical collapse ensues, presumably leading to a supernova event and, in at least some cases, the production of a neutron-star remnant. Although there are many remaining theoretical uncertainties (see, for example, refs. 12 and 13), a neutron star that is formed in this manner may well have a mass comparable to that of the core of the progenitor star (that is, $\sim 1.4M_{\odot}$). If a large fraction of observed neutron stars are actually found to have masses near this value, then substantial support for conventional pictures of advanced stellar evolution and neutron-star formation would have been obtained.

In the past few years, significant empirical information on the masses of several neutron stars has become available for the first time. These neutron stars are all in binary systems, and the mass information is derived from the observed interactions between the neutron stars and their companions. In this paper, we describe the present state of empirical knowledge of neutron star masses and discuss the theoretical implications of this information.

Binary systems containing neutron stars

At present, there are four known binary systems that contain X-ray pulsars and one system that contains a radio pulsar. (Six other X-ray pulsars have been discovered^{14–17} and these objects are also likely to be in binary systems, but neither conclusive evidence of binary membership nor useful orbital information is yet available.) For three of the binary pulsars, the pulse period (P) is well under 2 s, which is too short to be a plausible surface rotation period of a white dwarf (see for example, ref. 18) or the pulse period of a white dwarf that is vibrating in its fundamental mode¹⁹. Moreover, the existence of periodic pulsations rules out the possibility that any of these objects is a black hole. Thus, all three of these pulsars are almost certainly neutron stars. The remaining two objects (the X-ray pulsars 3U0900–40 (ref. 20) and Cen X-3 (refs 21, 22)) are also almost certain to be neutron stars, but the reasons for this conclusion are somewhat more complex and will be explained below.

Of course, it is also very likely that the remaining radio pulsars are all neutron stars. However, the neutron stars in binary systems are unique in that it is possible to place significant constraints on their masses that are independent of any theoretical assumptions concerning the equation of state of matter at high densities.

The existence of an object that emits periodic pulsations in a binary system enables one to measure the period, P_{orb} , and projected semimajor axis, a_p , of the orbit of the pulsar, from which one can infer the mass function:

$$f(M) = \frac{4\pi^2 (a_p \sin i)^3}{GP_{\text{orb}}^2} = \frac{M_c^3 \sin^3 i}{(M_p + M_c)^2}$$

where i is the inclination of the orbital plane with respect to the plane of the sky, M_p is the pulsar (neutron star) mass, and M_c is the mass of the companion star. To derive useful constraints on M_p , it is necessary to obtain independent information concerning the values of M_c , i , or both. The nature of this information is different for each of the five binary systems of interest.

In the following paragraphs, we describe the relevant observational properties of each of the five binary systems, the type of information used to constrain the value of M_p , and the indicated range of allowable values of M_p . Quoted error bars and confidence limits are taken from the original observational work, and wherever possible we state the level of significance. In all cases, the final limits on M_p are not formal confidence limits, but are rather the minimum and maximum values that are consistent with all of the available observational information.

3U0900–40 is an eclipsing binary system, as are all four

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X-ray pulsar binaries, with $P_{\text{orb}} \approx 8.966$ d (ref. 23). The binary is composed of an X-ray star and an optical companion, HD77581 (ref. 24), that has been classified as B0.51b (ref. 25). It has been discovered²⁰ that the X-ray star is a long period pulsar, with $P \approx 283$ s, $a_p \sin i = 111.4 \pm 3.3$ (1 σ) light s, and $f(M) = 18.46 \pm 0.79$ (1 σ) M_\odot (ref. 26). The orbit was also found to be slightly eccentric, with eccentricity $e \approx 0.13$ (ref. 26).

The apparent brightness of HD77581 ($V \approx 6.9$; ref. 27) makes it relatively easy to measure the optical radial velocity variations due to orbital motion. Hence, this system can, in effect, be treated as a double-line 'spectroscopic' binary. The optical radial velocities can be used to derive the projected semimajor axis ($a_c \sin i$) of the companion star, and one thereby obtains a second relation for the stellar masses in terms of measured quantities

$$M_p(a_p \sin i) = M_c(a_c \sin i)$$

Unfortunately, there are large non-statistical fluctuations in the optical radial velocities, probably caused by variations in the strong stellar wind from HD77581 (ref. 23) or other mass flow within the binary system. The smallest value of $a_c \sin i$ that has appeared in the literature is based on an analysis²⁶ of the most recent optical data obtained by van Paradijs *et al.*²⁸: $a_c \sin i = 7.79 \pm 0.74$ (1 σ) light s. On the other hand, the orbital fit to optical data that gives the largest value of $a_c \sin i$ and is still consistent with the known orbital eccentricity (which was recently determined accurately from X-ray data)²⁶ is solution 1a by Wallerstein²⁹: $a_c \sin i \approx 15.1$ light s.

The final constraints that one uses are the limits on $\sin i$ that can be derived from analysis of the ellipsoidal light variations of the companion star (which are caused by its rotation and its tidal distortion by the X-ray star) and the X-ray eclipse duration (which is also affected by the rotational and tidal distortion). The observed value of the eclipse duration is 1.90 ± 0.05 d (ref. 30). The most recent analyses^{31,32} indicate that $70^\circ \lesssim i \lesssim 90^\circ$ if $a_c \sin i \approx 7.8$ light s, and $80^\circ \lesssim i \lesssim 90^\circ$ if $a_c \sin i \approx 15.1$ light s. We note that one of these analyses³¹ correctly gave the range of acceptable values of $a_p \sin i$ before the measurement of this parameter from X-ray data, which lends credence to the standard theoretical picture of X-ray binary systems that is used in this method of analysis and to the results of similar analyses of other X-ray binaries described below.

Small values of $a_c \sin i$ and large values of i correspond to small values of M_p , and vice versa. Combining the measured mass function with the probable lower and upper limits on i and $a_c \sin i$, we obtain for the range of allowable values of M_p

$$1.4M_\odot \lesssim M_p \lesssim 3.5M_\odot$$

M_p is probably greater than the maximum stable mass for a white dwarf in the absence of differential rotation (internal rotation periods $\lesssim 10$ s; surface rotation period ≈ 283 s), and such strong differential rotation would probably induce a dynamical instability (compare refs 18, 33). For these reasons it has been concluded^{20,26} that this slow pulsar, as well as the fast pulsars discussed below, is almost certainly a neutron star.

The X-ray source **Cen X-3**, with $P \approx 4.842$ s, was the first X-ray pulsar to be discovered^{21,22}. The highly circular orbit ($e \lesssim 0.003$ (ref. 34)) of the X-ray star has $P_{\text{orb}} \approx 2.087$ d (ref. 22), $a_p \sin i = 39.73 \pm 0.03$ light s (ref. 22), $f(M) = 15.46 \pm 0.04 M_\odot$ (ref. 22), and an X-ray eclipse duration of 0.45 ± 0.02 d (refs 35, 36). A B0II star was established by Krzeminski³⁷ as the optical companion. The pulse period is not quite short enough to rule out a white dwarf model for this X-ray star, but its very high X-ray luminosity ($\sim 3 \times 10^{37}$ erg s⁻¹ (ref. 38)) strongly rules against white dwarf models³⁹. Hence, this object is again almost certain to be a neutron star.

When the measured ellipsoidal light variations^{37,40} of Krzeminski's star and the X-ray eclipse duration are incorporated into theoretical models for the tidal and rotational distortion of the optical star^{36,41,42}, the mass ratio ($q = M_p/M_c$) and i can be estimated: $0.04 \lesssim q \lesssim 0.09$ and $74^\circ \lesssim i \lesssim 90^\circ$. The acceptable values of q and i are strongly correlated. When these

constraints are combined with the measured mass function, limits on M_p are obtained^{36,41,42}

$$0.6M_\odot \lesssim M_p \lesssim 1.8M_\odot$$

Situated in the Small Magellanic Cloud, **SMC X-1** is the only extragalactic X-ray source known to be in a binary system⁴³. The optical companion, Sk160 (refs 44, 45), is of spectral type BOI (ref. 44). Recently, pulsations from the X-ray star with $P \approx 0.715$ s were discovered⁴⁶, followed shortly thereafter by a measurement of the orbital Doppler curve of the pulsar⁴⁷. The nearly circular orbit ($e \lesssim 0.04$ (ref. 47)) has $P_{\text{orb}} \approx 3.892$ d (ref. 48), $a_p \sin i = 53.83 \pm 0.35$ (1 σ) light s (ref. 47), $f(M) = 11.05 \pm 0.22$ (1 σ) M_\odot (ref. 47), and an X-ray eclipse duration of 0.60 ± 0.03 d (refs 43, 47, 48).

The projected orbital velocity amplitude ($v_c \sin i$) of Sk160 has not been well determined, but the available measurements indicate that $v_c \sin i \sim 40$ km s⁻¹ (ref. 49), corresponding to $a_c \sin i = (P_{\text{orb}}/2\pi) v_c \sin i \sim 7$ light s. Taking 20 and 60 km s⁻¹ as probable lower and upper limits on $v_c \sin i$, and using the observed X-ray eclipse duration in conjunction with theoretical models of the tidal and rotational distortion of the optical star to place constraints on the permissible values of i at each value of q , one obtains⁴⁷

$$1.1M_\odot \lesssim M_p \lesssim 4.0M_\odot$$

The ellipsoidal light variations of Sk160 have been measured⁵⁰⁻⁵⁴ but their analysis is complicated by the effect of X-ray heating of the optical star, which is appreciable in this system. Estimates of q and i based on such analyses^{55,56} are consistent with those obtained from the considerations described in the preceding paragraph.

Hercules X-1 is unique among all X-ray binaries in that the companion star, HZ Herculis⁵⁷⁻⁶⁰, is known to be of relatively late spectral type. When the X-ray star is in eclipse, the spectral type is late A or early F; but when the X-ray star is visible, the optical luminosity increases by a factor of ~ 4 and the spectral type changes to B (refs 58, 61-65). These optical variations are apparently caused by the strong heating of one face of the optical companion by the X-ray star. Unfortunately, the uneven surface brightness greatly complicates the extraction of mass information from the measured optical radial velocities, since these velocities are consequently affected strongly by the stellar rotation as well as the orbital motion^{62,66}.

The strong X-ray heating also precludes observation of the ellipsoidal light variations at the present time. However, from photographic plates dating back to 1890, it has been found⁶⁷ that the X-ray star apparently ceases its emission for extended periods lasting months to years, during which HZ Her displays ellipsoidal variations. The number and quality of the old photographic plates are insufficient to place useful constraints on q and i (refs 42, 66), but observations of future inactive-state ellipsoidal variations should be very powerful in this regard.

The binary system has $P_{\text{orb}} \approx 1.700$ d (refs 68, 69), a nearly circular orbit ($e \lesssim 0.002$; ref 34) and an X-ray eclipse duration of 0.24 ± 0.01 d (ref. 69), and the X-ray pulsar has $P \approx 1.238$ s (ref. 68, 69), $a_p \sin i = 13.18 \pm 0.03$ light s (ref. 68), and $f(M) = 0.85 \pm 0.01 M_\odot$ (ref. 88). Estimates of M_p have been made⁶⁶ using this eclipse duration and mass function together with theoretical models of the tidal and rotational distortion of HZ Her. One obtains $q \lesssim 0.7$, no meaningful constraint on i , and

$$M_p \lesssim 1.8M_\odot$$

Other methods of estimation of M_p give values that are consistent with this range but are unable, at present, to constrain more strictly the range of allowable values^{42,66}.

Recently, another independent estimate of M_p has been carried out⁷⁰, based on an analysis of extensive observations of 1.24-s optical pulsations that are occasionally seen from the system and apparently result from the reprocessing of pulsed X rays near the surface of HZ Her. In the context of the

assumed model, it was found that HZ Her must fill its critical lobe and a value of $M_p = 1.30 \pm 0.14 M_\odot$ was thereby obtained. However, it is uncertain at present how sensitive this result is to some of the assumptions of the model, including the assumption that HZ Her is in synchronous rotation and the implicit assumption that there is no strong orbital-phase-dependent obscuration of the optical pulsations by other matter within the binary system. At least one further analysis of the optical pulsations is being carried out at present (T. Chester, personal communication).

PSR1913+16 is the only radio pulsar (of ~ 150) that is known to be in a binary system. The pulsar, which has $P \approx 0.0590$ s, was found to be in an eccentric binary orbit with $e \approx 0.62$, $P_{\text{orb}} \approx 0.3230$ d, $a_p \sin i \approx 2.336$ light s, and $f(M) \approx 0.1313 M_\odot$ (refs 71, 72). No eclipses are observed in this system⁷¹.

During subsequent observations over the course of about a year, it was discovered that the longitude of periastron (related to the angle between the view direction from the earth to the star and the line of closest approach from the star to its companion) was changing at a rate of $\dot{\omega} = 4.22 \pm 0.04^\circ \text{ yr}^{-1}$ (ref. 72). This effect, known as apsidal motion, can be completely accounted for by general relativity. However, it is possible, depending on the radius and other properties of the unseen companion, that some or all of the apsidal motion could be caused by a tidally or rotationally induced quadrupole moment of the companion^{73,74}. Under the assumption that the observed apsidal motion is entirely a general relativistic effect, the measured mass function can be combined with the general relativistic expression for this effect to obtain a relation for the sum of the two stellar masses^{72,75}

$$M_p + M_c = 2.83 M_\odot$$

If some of the apsidal motion is due to a tidally induced quadrupole moment, or to a rotationally induced quadrupole moment with the axis of rotation at least $\sim 35^\circ$ out of the orbital plane⁷⁵, then $M_p + M_c$ must be less than this value.

A normal main-sequence star would induce a value of $\dot{\omega}$ much larger than that observed^{73,76,77}. The companion could be a white dwarf, neutron star, or black hole, or it might be a peculiar star that is nondegenerate but nonetheless has an anomalously small radius ($\lesssim 0.1 R_\odot$). Many theoretical studies of such stars have appeared in the literature (see, for example, refs 78–81).

Combining the sum of the masses with the measured mass function, one obtains constraints on the allowable values of M_p (ref. 75). If the companion is a white dwarf, then its mass must be less than $\sim 1.4 M_\odot$ (unless it is undergoing rapid rotation) and this yields $1.4 M_\odot \lesssim M_p \lesssim 1.9 M_\odot$. In the case where the unseen companion is also a neutron star, then $M_p \lesssim 1.9 M_\odot$ and the mass of the companion neutron star is also constrained: $1.0 M_\odot \lesssim M_c \lesssim 2.9 M_\odot$. Moreover, at least one of the two neutron stars must have a mass $\gtrsim 1.4 M_\odot$. For other possible types of companion stars, all that can be determined is that $M_p \lesssim 1.9 M_\odot$.

In summary, as long as the theory of general relativity is correct, one obtains:

$1.4 M_\odot \lesssim M_p \lesssim 1.9 M_\odot$ for a normal white-dwarf companion;
 $M_p \lesssim 1.9 M_\odot$ for other types of companion.

Summary and conclusions

At present, there are five X-ray and radio pulsars that are very probably neutron stars and that are contained within binary systems. The periodic pulsations from these pulsars and their interactions with their respective companion stars enable constraints to be placed on the neutron-star masses. The available constraints are summarised in Fig. 1.

It is apparent that these mass estimates are all still entirely consistent with the predictions of nuclear physics. None of these neutron stars need have a mass greater than $\sim 1.4 M_\odot$, while a minimum mass of $\sim 1.8 M_\odot$ would be needed to exclude at least

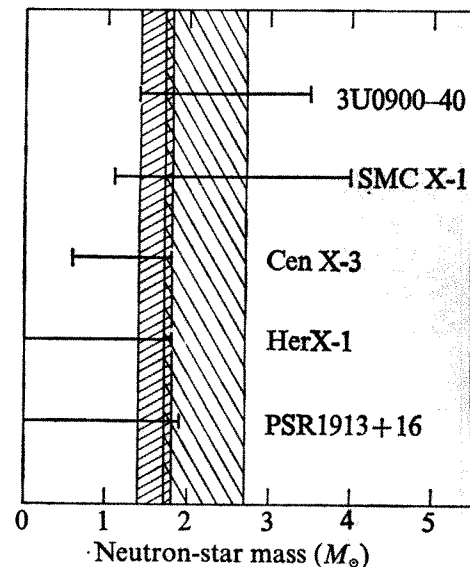


Fig. 1 Empirical constraints on the masses of neutron stars. For each of the five binary systems containing neutron stars, the bar denotes the range of masses that is consistent with all of the available observational information. The close-hatched region ($1.4\text{--}1.8 M_\odot$) represents the range of masses consistent with all five neutron stars. The open-hatched region ($1.7\text{--}2.7 M_\odot$) indicates the range of limiting neutron-star masses predicted by present conventional theories of many-body nuclear physics (see text).

some of the equations of state that have been derived^{2–4} from present conventional theories of many-body nuclear physics.

On the other hand, if one hypothesises that most or all neutron stars have about the same mass, then the range of masses that are allowable for all five cases is quite narrow ($\sim 1.4\text{--}1.8 M_\odot$; Fig. 1). It is noteworthy that this is consistent with the range of masses that one might expect if neutron stars are formed in supernova events resulting from the collapse of the degenerate cores of highly evolved stars.

For 3U0900–40, Cen X-3 and SMC X-1, significant refinement of the neutron-star mass estimates must probably await extensive new observations of the optical radial velocities and an improved theoretical understanding of systematic fluctuations in the radial velocities and other measured properties of the companion stars. In the case of Her X-1, further analysis of the optical pulsations is promising as an improved method of mass estimation. In addition, when Her X-1 enters another extended X-ray inactive state, measurements of the radial velocities and ellipsoidal light variations of the optical companion should greatly refine the mass estimate of the neutron star. Further refinements of the neutron-star mass estimate of PSR1913+16 will be possible when additional mass-dependent relativistic effects (gravitational redshift and second-order Doppler shift) can be separated from the first-order orbital Doppler shift; this will occur when the longitude of periastron advances through an appreciable angle from its present value, which will require $\gtrsim 5$ yr (ref. 82).

Several other X-ray pulsars have recently been discovered and it seems likely that most or all of these objects are in binary systems. For at least two of these objects, 3U0352+30 and A0535+26, there is already some direct observational evidence of binary membership^{83–85}. As additional pulsars are discovered and shown to be in binary systems, and as constraints on their masses are developed, the improved observational statistics will constitute a further refinement in empirical information on neutron-star masses.

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articles

Polarisation detection at radio wavelengths in three spiral galaxies

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Polarisation at radio wavelengths has been measured in the disk radiation of M51, M81 and M31 using the Westerbork Synthesis Radio Telescope. The magnetic field of M51, as implied by the polarisation vector map, is oriented preferentially in the tangential direction. The intrinsic polarisation of the disk radiation of M51 is estimated to be, on average, 12%, corresponding to an anisotropy (or degree of uniformity) in the magnetic field of 14% with scale length ≤ 3 kpc. A 7% lower limit is set on the disk polarised radiation of M81, but on some parts of the disk of M31 it reaches 20%.

WE have constructed a model which is especially suitable for the interpretation of radio polarisation measurements on the disks of spiral galaxies—polarisation is evidence for anisotropy in the magnetic field. Models which relate, under various conditions of field topology and object geometry, the observed linear polarisation and a measure of the anisotropy of the field have been constructed by Korchakov and Syrovatskii¹ and by Burn², but neither of these models is quite applicable to the case we discuss, although, for the conditions of interest, the results do not seem to be very model dependent.

We shall consider a region in a disk galaxy defined, of necessity, by the angular resolution of the observations (see Table 1). This region is thought of as divided into volume elements of equal size, so small that the magnetic field within

each element is essentially homogeneous and unidirectional. We now represent the distribution of magnetic fields by supposing that a fraction f of the volume elements contains a field with a single uniform spatial orientation (\mathbf{u}), while the orientation of the field in the remaining elements is randomly distributed (\mathbf{r}). We further assume that the distributions of relativistic electron densities and magnetic field strengths are the same in both components. If this is not the case, then the contributions to the observed intensity from the two components must be weighted according to their mean volume emissivities, $\langle n_{\text{rel}} H^{(\gamma+1)/2} \rangle$, where γ is the electron energy spectral index. The fraction f is a measure of the anisotropy of the magnetic field within the resolution element. Using standard expressions³, it is easily shown that under the above conditions the degree of intrinsic polarisation is

$$\rho = \frac{3\gamma+3}{3\gamma+7} \left[1 + \frac{(1-f)\pi^{1/2}\Gamma[(\gamma+5)/4]}{2f(\sin \theta)^{(\gamma+1)/2}\Gamma[(\gamma+7)/4]} \right]^{-1} \quad (1)$$

where θ is the angle between the line of sight and $\hat{\mathbf{u}}$.

The observations described in this paper indicate that the orientation of \mathbf{u} , in M51 at any rate, is largely tangential in the galactic plane. We shall make only the more general assumption that \mathbf{u} is parallel to the disk and, taken over the galaxy as a whole, has no further preferential orientation with respect to

and i is the angle between the plane of the sky and the galactic plane. In Fig. 1, $\langle \rho \rangle$ is shown as a function of f for $\gamma = 2.5$ and for two values of i .

Two points should be stressed: first, the degree of anisotropy, f , refers to the angular resolution element. We expect that it will increase with decreasing beam size; second, no account has been taken of depolarisation from either differential Faraday rotation along the line of sight or from the changes in rotation measure with position within a resolution element. This will often be of importance, particularly in regions of high density, and thus the value of the anisotropy derived by this method will be a lower limit. At $\lambda = 6$ cm, however (see below), we do not expect Faraday depolarisation to be very effective.

Observations and results

The WSRT has been used to observe M51, M81 and M31. The telescope and reduction procedures are described elsewhere⁴⁻⁸. The observational parameters for each of the maps presented hereafter are given in Table 1. The system noise at 1,415 MHz has been reduced by a factor of ~ 2.5 since earlier observations of the same objects with the WSRT.

M51

M51 has been observed with the WSRT at wavelengths of 6, 21 and 49 cm. No polarisation was detected at 49 cm implying

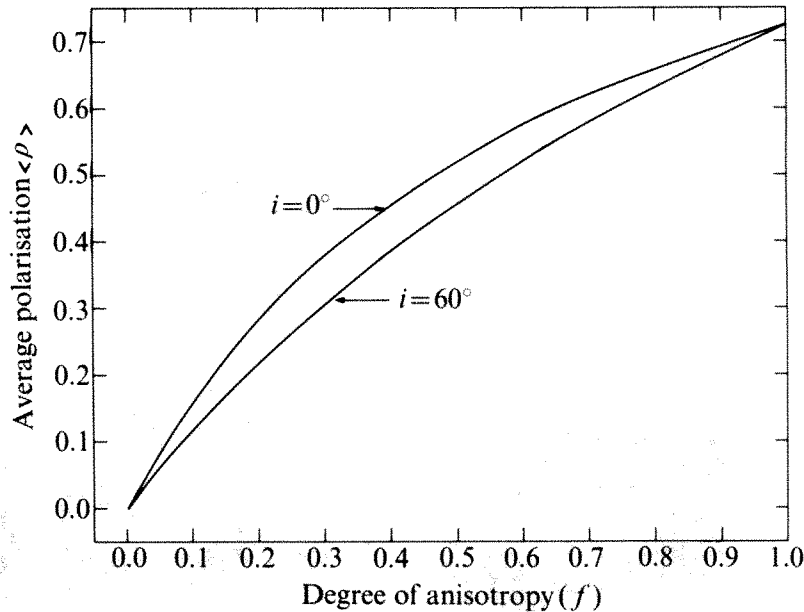


Fig. 1 The average intrinsic degree of polarisation, $\langle \rho \rangle$, plotted as a function of the degree of anisotropy of the magnetic field, f , for electron energy spectral index $\gamma = 2.5$ and two values of the galactic inclination, i .

any fixed direction in space. Clearly a tangential field belongs to this class. In this case, the average degree of polarisation (the scalar sum of the polarised flux from all resolution elements divided by the total flux) is given in equation 1 with $(\sin \theta)^{(\gamma+1)/2}$ replaced by its average value

$$\langle \rho \rangle = \frac{3\gamma+3}{3\gamma+7} \left[1 + \frac{(1-f)\pi^{1/2}\Gamma[(\gamma+5)/4]}{2f\Gamma[(\gamma+7)/4]F(i)} \right]^{-1} \quad (2)$$

where

$$F(i) = \frac{1}{2\pi} \int_0^{2\pi} (1 - \sin^2 i \sin^2 \phi)^{(\gamma+1)/4} d\phi$$

$$= 1 + \sum_{n=1}^{\infty} \frac{(-1)^n}{n! 4^n} \sin^{2n} i \prod_{j=1}^n \frac{(\gamma+5-4j)(2j-1)}{2j} \quad (3)$$

a 3σ upper limit on the polarised brightness temperature of 1.5 K. The beam HPBW of the synthesised 49-cm beam is $56'' \times 76''$, or 2.6×3.6 kpc at an assumed distance of 9.7 Mpc (ref. 9).

Polarisation at 6 cm

The 6-cm observations of M51 have been described elsewhere¹⁰. To increase the signal to noise ratio, the observations were convolved to a $56'' \times 76''$ beam and two maps were produced: an intensity map and a polarisation map (Fig. 2). No appreciable Faraday rotation is expected at such a short wavelength in view of the residual polarisation which is observed at 21 cm (see below). We find it significant, therefore, that at a level $> 2.5\sigma$ ($\sigma = 0.009$ K = 0.9 mJy/beam) the polarisation vectors are generally perpendicular to the spiral arms. This suggests an approximately tangential magnetic field structure. For the Galaxy, such a field configuration is referred to as 'longitudinal' (as opposed to helical). In a recent paper,

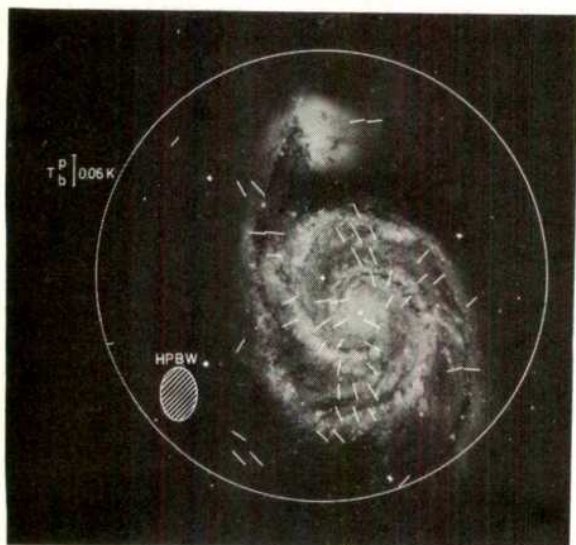


Fig. 2 The polarisation vectors of M51 at 6 cm. Vectors whose amplitude is < 2.5 mJy per beam ($\sim 2.5\sigma$) are not drawn. The polarised flux is not corrected for the primary beam attenuation. The primary beam half power circle is drawn as a continuous line.

Spelstra and Brouw (in preparation) argue that a longitudinal field also fits the radio polarization observations of the Galaxy better than a helical field. A tight-helix field configuration would result in a net polarisation along the spiral arm. Such a configuration is ruled out by our results. Our observations do not exclude, though, a very-open-helix field configuration.

The location of the polarised brightness peaks, which range up to ~ 0.05 K, cannot be precisely determined because of the large beam and the weak signal. The percentage polarisation at a few points was computed by dividing the polarised flux by the intensity deduced from the preliminary MPIfR 100-m dish observations of M51 at 4.8 GHz (Wielebinski, private communication). Peaks as high as 20% were found. To determine the average polarisation, we have analysed the distribution of the observed polarised signal to derive the polarised flux (the scalar sum over all resolution elements) corrected statistically for noise. A similar correction has been

applied to all polarisation data. The corrected polarised flux was divided by the total flux within the same region after subtracting a point source of 15 mJy at the nucleus. We find an average polarisation of 12% for radii < 9 kpc (limited by the off-centre instrumental polarisation). Because of the short wavelength, this is probably characteristic of the intrinsic polarisation. This implies an anisotropy of the magnetic field, f_c of 14% on the scale of the resolution element (see Fig. 1; the inclination of M51 is taken to be 35°).

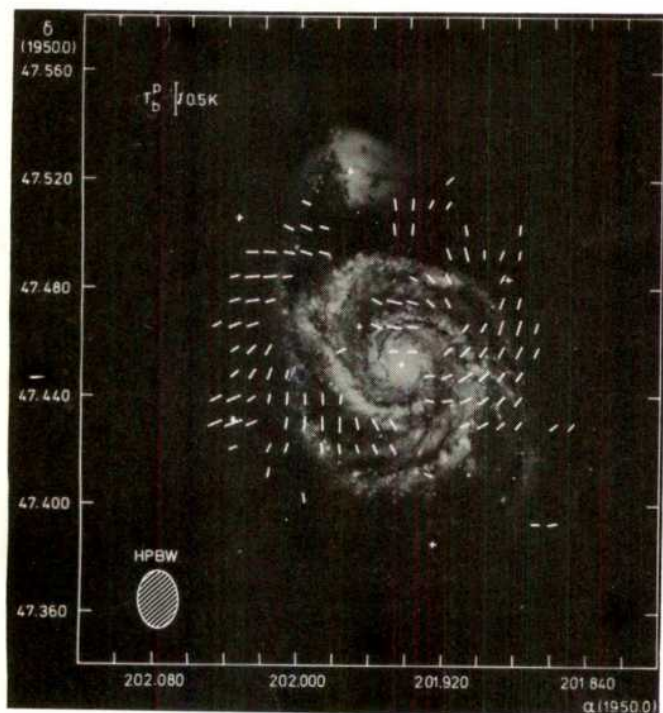


Fig. 3 The polarisation vectors of M51 at 21 cm. Vectors whose amplitudes are < 1 mJy per beam (0.12 K $\approx 2.5\sigma$) are not drawn.

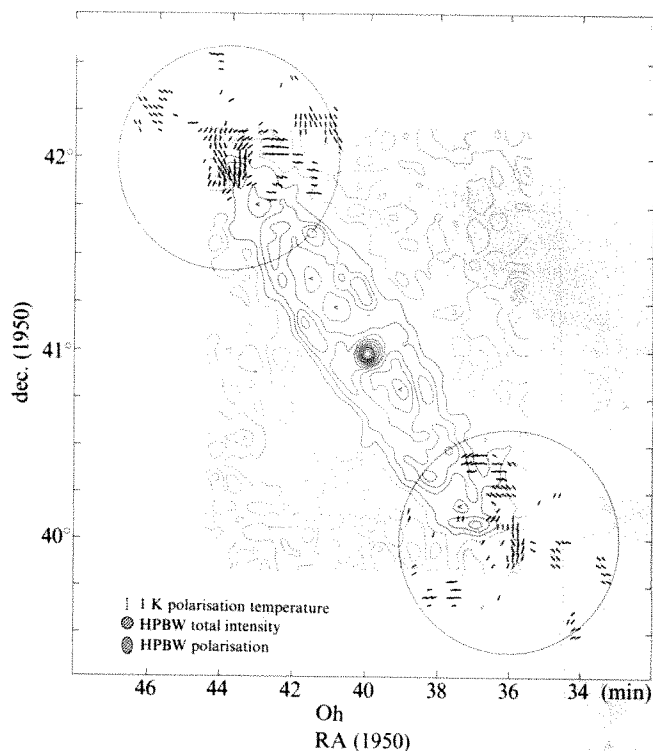


Fig. 4 The polarisation vectors of M81 at 21 cm. Vectors whose amplitudes are < 1.3 mJy per beam (≈ 0.05 K $\approx 3\sigma$) are not drawn. The polarised flux is not corrected for the primary beam attenuation. The polarisation vectors at the western edge of the photograph are related to an external source and may partly arise from instrumental polarisation.

Polarisation at 21 cm

New 21-cm observations of M51 have recently been carried out with the WSRT and will be described elsewhere (Segalovitz, in preparation). Polarisation at the 3σ level ($\sigma = 0.25$ mJy/beam = 0.17 K, determined empirically) was detected at a few scattered locations in the full resolution map ($\text{HPBW}_x \times \text{HPBW}_y = 24'' \times 32'' = 1.1 \times 1.5$ kpc). These peaks did not in general coincide with regions of maximum intensity. They are several times weaker than the polarisation peaks reported previously¹¹.

Fig. 5 Polarisation vectors at 49 cm in two fields in M31. Only amplitudes > 0.34 K ($\approx 5\sigma$) have been plotted. The two circles correspond to a primary beam attenuation of 0.67. With few exceptions, only points lying inside these circles have been plotted. The amplitudes have not been corrected for primary beam attenuation. The polarisation vectors are shown superposed on the 408 MHz total intensity map of Pooley¹⁵. The contour interval is 3 K, corresponding to ~ 1 K at 610 MHz, and the outermost solid curve is the first contour above the nominal zero level. Because of the missing short spacings, the true zero level is uncertain.



Convolving to a $56'' \times 76''$ beam (and thus increasing the signal-to-noise ratio) revealed an extensive distribution of polarised radiation (Fig. 3). The polarised flux over a large part of the Galaxy is at a general level of 1.25 mJy/beam ($= 0.15$ K $\approx 3\sigma$) with peaks at the 0.21-K level. The strongest peak (0.27 K) is associated with an external source E of M51. About half of the area from which the polarised radiation comes is not located at intensity peaks, and for this the resolution is not good enough to make a definite statement. The tendency of the polarisation peaks to be found away from the intensity peaks may result from a less ordered magnetic field or stronger Faraday depolarisation in regions of higher density. The same tendency is found in strong extragalactic radio sources^{12,13}.

In most regions the polarisation vectors are systematically rotated by $\sim 45^\circ$ counterclockwise from the radial direction. Vallée and Kronberg¹⁴ estimate a galactic Faraday rotation in the direction of M51 of -10 ± 15 rad m^{-2} , corresponding to a clockwise rotation of $25 \pm 37^\circ$ at 21 cm. This estimate is not consistent with our data (from comparison of the directions of the 6-cm and 21-cm polarisation vectors). Small-angle deviations of this magnitude from the values predicted from their model are not, however, unexpected. We conclude, from the uniformity of the polarisation vector rotation, that the

systematic Faraday rotation over the resolution element in M51 is at most of comparable magnitude to that estimated in the Galaxy ($\lesssim 20$ rad m^{-2}).

A polarisation map was made by dividing the polarised flux by the total intensity at points where the polarised flux was above the 3σ level. Polarisation peaks reach values of 20%. The average polarisation percentage was derived by dividing the total polarised flux by the total intensity, and was found to be 2.5% for radii < 12 kpc. In this calculation, the flux of the nucleus of M51 (35 mJy) was first subtracted from the total flux. Comparing this value with the apparently intrinsic 12% polarisation, we get a rough estimate of 50 rad m^{-2} for the r.m.s. variations in rotation measure on angular scales much smaller than the resolution element of $56'' \times 76''$ (using the model of Burn²) as compared with the upper limit of 20 rad m^{-2} for large scale fluctuations.

M81 at $\lambda = 21$ cm

The disk of M81 has been observed with the WSRT at 21 cm and at 49 cm. A detailed account of the observations will be given elsewhere (Segalovitz, in preparation). The 49-cm observations show no polarisation > 2.5 K (3σ). The 21-cm

Table 1 Instrumental and observational parameters

Galaxy	M51	M51	M81	M31
Wavelength (cm)	6.0	21.2	21.2	49.2
1 K is equivalent to... (mJy per beam)	103	8.24	27.9	19
R.M.S. noise* (mJy per beam; K)	0.9; 0.0088	0.4; 0.48	0.44; 0.016	1.2; 0.06
Observing dates (day/month/year)	25-27/12/74	7,21/6/75	10,19/6/75	15,20,25,26/11/75
	1-6/1/75	5/7/75	30/7/75	3,5,9,10/12/75
	8,17/1/75	7/8/75	3,19/8/75	
Observing time (h)	12 \times 12	4 \times 12	4 \times 12	2 \times 4 \times 12
Baseline coverage: from, to, by (m)	36,180,18	36,612,18	36,306,18	36,378,18
Primary beam, HPBW'	11	36	36	83
f.w.h.m. of synthesised and restoring beam (RA'' \times dec'')	56 \times 76	56 \times 76	114 \times 126	214 \times 326
HPBW of synthesised and restoring beam (linear size in the galactic plane, kpc)	3.2 \times 3.6	3.2 \times 3.6	3.6 \times 2.0	2.4 \times 1.0
Scale corresponding to shortest baseline'	5.7	20.2	20.2	47
Position of field centre (RA ^(°) ; dec ^(°))	201.9666;47.4666	201.9666;47.4666	147.92;69.22	9.0;40.0 and 11.0;42.0†
No. of figures in this paper	2	3	4	5

*The values refer to the Q, U and I maps.

†Centres of two fields (see text).

observations convolved to a $1.9' \times 2.1'$ beam (1.8×2 kpc at a distance of 3.2 Mpc) reveal polarisation at a level of 0.05 K to 0.07 K ($\sigma = 0.016$ K = 0.44 mJy per beam) as shown in Fig. 4. The polarisation direction shows no preferential orientation with respect to the azimuthal direction at the few points where it can be determined. Polarisation percentages were derived by dividing the polarised flux by the intensity at some points where a comparatively high polarised flux was detected. The total flux densities were taken from the corresponding intensity map after adding a constant 0.035 K to correct approximately for flux in large scale features which is not detected by the Westerbork telescope. Peaks at the 20% level were found. The average polarisation, derived by dividing the total polarised flux by the total flux after subtracting the nucleus, was 7%. This is considerably higher than for M51. The difference may arise from a smaller Faraday depolarisation in M81 or to a more ordered magnetic field. Taken at face value, the average polarisation of 7% implies that 10% of the magnetic field is ordered (see above and Fig. 1; the inclination of M81 is taken to be 59°). Since Faraday depolarisation inside the Galaxy may be appreciable at 21 cm, the above value should be regarded as a lower limit.

M31 at $\lambda = 49$ cm

A survey of M31 at $\lambda = 49$ cm (see Table 1) has recently been completed. The results will be published in full elsewhere. Examination of the first reductions shows widespread linear polarisation, and a sample of these preliminary results, derived from two fields centred on the major axis and $80'$ on either side of the nucleus, is illustrated in Fig. 5. (Observations of the central region have not yet been fully reduced.) The polarisation vectors have been derived from maps convolved to a $3.6' \times 5.4'$ beam. Only vectors whose amplitudes are $\geq 5\sigma$ (see first note in Table 1) have been plotted. Since the maps of total intensity, whose preparation is much more elaborate on account of the multitude of background sources and the missing short spacings, are not yet available, the polarisation vectors have been superimposed on Pooley's 408-MHz ($\lambda = 73$ cm) map of M31 (ref. 15), which corresponds to approximately the same angular resolution ($4' \times 4'$). Assuming a spectral index of $\alpha = 0.8$ ($S \propto \nu^{-\alpha}$), the contour interval of 3 K on the 408-MHz map would become ~ 1 K on a corresponding map at 610 MHz. Pooley's observations were carried out with the E-vector

of the feed in position angle 90° and, depending on the polarisation, may not be representative of the unpolarised intensities at specific points. Moreover, because of the missing short intervals, the nominal zero may correspond to a true brightness temperature of as much as one contour interval above zero. Three other radio continuum surveys¹⁶⁻¹⁸, at various frequencies and resolutions, all indicate typical brightness temperatures at 49 cm of 1-2 K in these regions.

As in the case of M51, the regions of maximum polarised brightness temperature do not generally coincide with peaks of intensity. While the maximum degree of polarisation is as yet difficult to estimate because of the uncertainty in the zero level, it must amount to several tens of per cent in some regions. Even where the total intensity is relatively high, and correspondingly less uncertain, degrees of polarisation $> 20\%$ are found. A quantitative discussion of these results must, however, await complete reduction of the WSRT observations. Although we might expect quite large Faraday rotation at this frequency, the polarisation vectors show considerable consistency in orientation locally and tend to maintain a constant orientation with respect to the spiral pattern. This suggests that for the regions of M31 where polarisation is detected, the internal Faraday rotation is small compared to M51 and M81.

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Cytoskeletal control of surface membrane mobility

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Cytochalasin B induced redistribution of actin microfilaments into a bulge of the cytoplasm, bringing cell-surface elements to the corresponding surface area. This process was reversible, inhibited by colchicine and enhanced by antibodies to cell-surface antigens.

THE mechanisms responsible for the organisation of the surface membrane¹ and for the mobility of molecules within it are not understood. Long- but not short-range movements of surface antigens²⁻³ are dependent on cell metabolism^{2,9,10}, and inhibited by cytochalasin B (CB)^{7,11}, but not by colchicine¹¹. CB is thought to disrupt a micro-

filament system, containing actomyosin, located below the surface membrane¹². It has therefore been proposed that redistribution of cell surface molecules depends on microfilament activity⁷. In most studies, however, CB has affected membrane mobility only partially^{2,7,10,11} and since the compound has many other effects it may influence membrane mobility in several ways not necessarily involving microfilaments. In addition, CB induces morphological effects on the surface membrane, suggesting that this is one site of action of the compound¹¹.

Microtubular activity has been linked with ligand-induced redistribution (capping) of membrane components in lymphocytes. Capping of immunoglobulin (Ig) on mouse lymphocytes is inhibited by concanavalin A (con A) in

large doses. This inhibition is abolished by colchicine¹⁵⁻¹⁷, which disrupts microtubules. Colchicine also augments the inhibitory effect of CB on capping¹¹. Con A-capping in rabbit ovarian granulosa cells redistributes cytoplasmic microtubules¹⁸.

Here we present evidence for an interaction of cell-surface molecules with the submembranous microfilament system of actomyosin and with the microtubules. In three test systems CB induced a simultaneous reversible redistribution of cell-surface molecules and submembranous actin microfilaments which was counteracted by colchicine.

The cells studied were uninfected, and measles virus (Edmonston strain)-infected heteroploid human lung fibroblasts of the strain Lu 106, mouse L-cells, mouse

lymphocytes, normal human lymphocytes and cells of two human lymphoblastoid cell lines of B cell origin. The antigenic specificities examined included measles virus haemagglutinin (HA), H-2, β_2 -microglobulin (β_2 -m), species antigens (rabbit anti-Lu 106), and Ig on the appropriate cell types.

CB-induced redistribution

Cells were first incubated for 60 min in spinner culture in Eagle's spinner medium supplemented with 5% foetal calf serum (FCS), and then for a further 60 min in the presence of CB ($10 \mu\text{g ml}^{-1}$) unless otherwise stated. Thereafter, to

Fig. 1 *a*, Untreated Lu 106 cells. Indirect IF-staining detecting β_2 -m. *b*, Lu 106 cells treated with CB. Polarisation of blebs. Indirect IF-staining detecting β_2 -m. *c*, Lu 106 cells treated with CB plus colchicine. Blebs distributed all around the cell surface. Indirect IF-staining detecting β_2 -m. *d*, Lu 106 cells treated with CB. Discontinuous IF-staining detecting β_2 -m. Indirect technique. *e*, Chronically infected Lu 106 cells treated with CB. Caps. Indirect IF-technique detecting measles virus haemagglutinin. *f*, Chronically infected Lu 106 cells kept in 0.1% DMSO. Virtually continuous IF-staining. Indirect IF-technique detecting measles virus haemagglutinin.

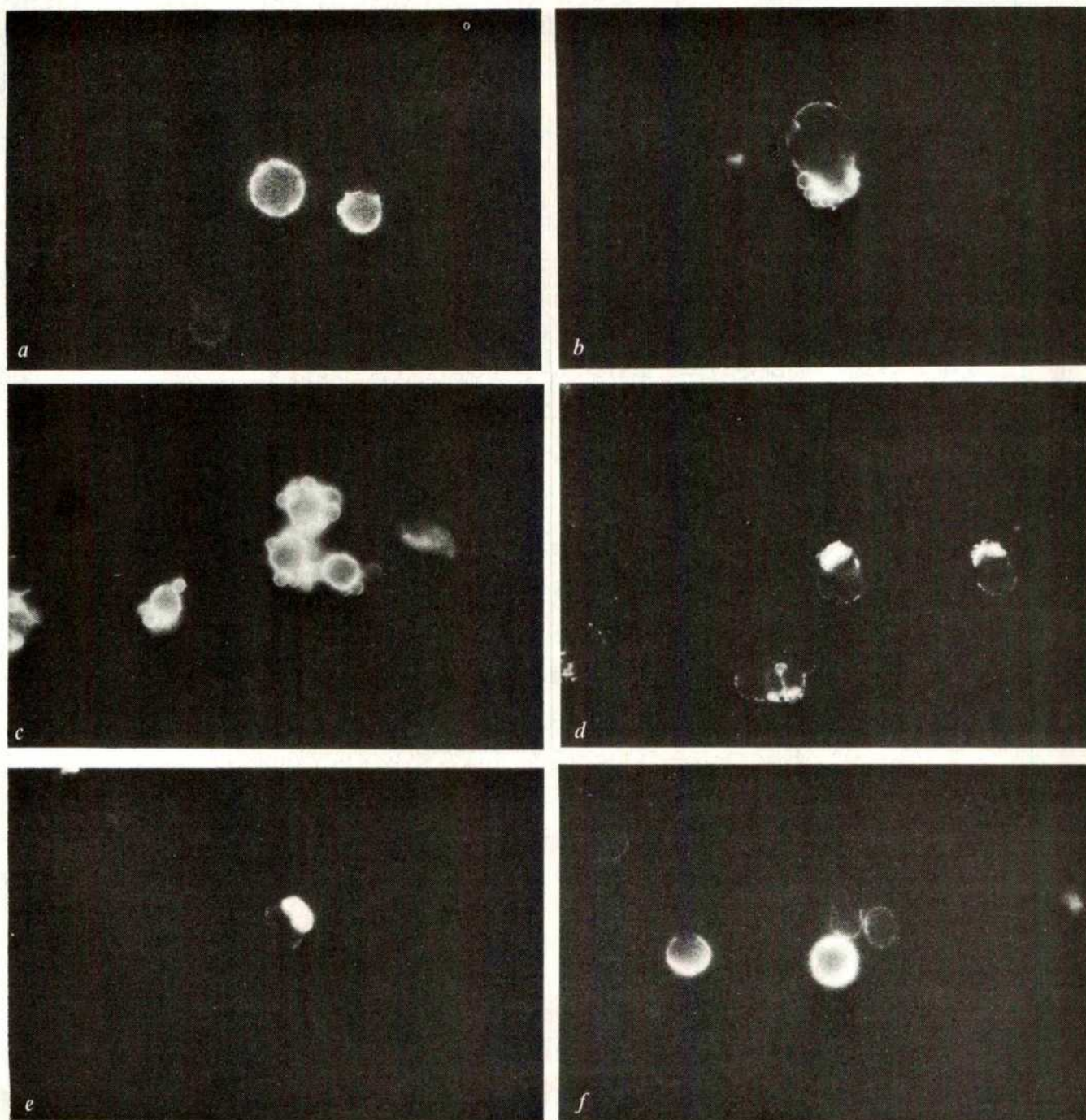


Table 1 *a* Effects of cytochalasin B on cell morphology

Cells	Antiserum against	Treatment					
		None	CB	CB+Colchicine	Colchicine	Antibodies	CB+Antibodies
Carrier	Ha	Round cells	IF-neg blebs at uropods	IF-neg blebs around the cell	Round cells	Round cells	IF-neg blebs at uropods
	β_2 -m	Round cells	IF-pos blebs at uropods	IF-pos blebs around the cells	No blebs	No blebs	IF-pos blebs at uropods
	Species antigens	Round cells	IF-pos blebs at uropods	IF-pos blebs around the cells	No blebs	No blebs	IF-pos blebs at uropods
Lu 106	β_2 -m	Round cells	IF-pos blebs at uropods	IF-pos blebs around the cells	Round cells	Round cells	IF-pos blebs at uropods
	Species antigens	Round cells	IF-pos blebs at uropods	IF-pos blebs around the cells	No blebs	No blebs	IF-pos blebs at uropods
L-cells	H-2	Round cells	IF-pos blebs at uropods in 70% of cells	Uropods in 30% of cells (partial inhibition)	Round cells	Round cells	Round cells

b Effects of cytochalasin B on antigen distribution at the cell surface

Cells	Antigen	Treatment					
		None	CB	CB + Colchicine	Colchicine	Antibodies	CB + Antibodies
Carrier	HA	Confluent	Caps	Confluent	Confluent	Discontinuous, caps	Pronounced capping
	β_2 m	Confluent	Discontinuous, caps	Confluent	Confluent	Discontinuous, few caps	Pronounced capping
	Species antigens	Confluent	Discontinuous	Confluent	Confluent		
Lu 106	β_2 m	Confluent	Discontinuous, caps	Confluent	Confluent	Discontinuous	Pronounced capping
	Species antigens	Confluent	Discontinuous	Confluent	Confluent		
L-Cells	H-2	Confluent	Discontinuous, polarised to the uropod	Confluent	Confluent		

The cells were incubated for 60 min at 37 °C in Eagle's spinner medium supplemented with 5% FCS before further treatment. The cells, 2×10^6 cells in 1 ml per tube, were then incubated further for 60 min *a*, with no treatment; *b*, in the presence of $10 \mu\text{g ml}^{-1}$ of CB; *c*, in the simultaneous presence of $10 \mu\text{g ml}^{-1}$ of CB and 10^{-2} M of colchicine (colchicine was added 30 min before CB); *d*, in the presence of 10^{-2} M colchicine, and subsequently PFA-fixed before IF-staining. When antibodies were present during the experiment, the cells were reacted with antiserum and conjugate, respectively, for 30 min + 30 min before CB-incubation in 1 ml at 37 °C for 60 min, followed by PFA-fixation. Carrier cells and Lu 106 cells were stained with a rabbit anti-HA serum, a rabbit anti- β_2 -microglobulin serum, a rabbit anti-HeLa serum, a monkey anti HeLa serum, respectively. The L-cells were stained with mouse ACA anti-A/SN and C57BL anti-C3H, respectively.

prevent further redistribution, cells were fixed in 4% paraformaldehyde for 10 min at 4 °C. Finally, antigens were detected on the surface membrane by direct or indirect immunofluorescence staining for 30 min or 30+30 min respectively, at 0-4 °C.

The morphology of Lu 106 and L-cells was affected by CB (Fig 1, Table 1*a*). Cells exposed to CB often formed a bulge which looked like a uropod¹⁹ (Fig. 1). These were most frequent on L-cells, occurring in 80% of cases. In addition, cells treated with CB had 5-15 blebs on the part of the cell carrying the "uropod". β_2 -m and species-specific antisera reacted strongly with the blebs whereas the HA-specific antiserum did not react.

The distribution of antigens at the cell surface was also affected by CB (Fig. 1, Table 1*b*). After incubation in the presence of CB the measles virus HA was present as "caps" on the "uropods" of 40% of infected cells. Control cells incubated without CB, or in the presence of 0.1% DMSO, had a ring-type distribution of HA at the cell surface (Fig. 1). β_2 -m and species antigens of both infected and uninfected Lu 106 cells were also redistributed, less, however, than the HA, and some of these antigens were unaffected.

In CB-treated L-cells, reacted with anti-H-2 sera (ACA anti-A and C57BL anti-C3H/K), cell surface antigens were redistributed in the direction of the "uropod". The extent of redistribution was similar to that of β_2 -m and species antigens on Lu 106 cells. Attempts to induce redistribution of surface antigens by CB in normal lymphocytes of mouse and human origin and in human lymphoblastoid cell lines failed.

Concentrations of CB between 1 and $5 \mu\text{g ml}^{-1}$ were necessary to achieve movement of HA on measles-infected cells, and above $10 \mu\text{g ml}^{-1}$ redistribution reached a plateau (Table 2). While $5 \mu\text{g ml}^{-1}$ was enough for some cap formation of HA, redistribution of β_2 -m required $10 \mu\text{g ml}^{-1}$.

To see whether the induced redistribution of antigens was reversible, CB-treated cells showing redistribution were kept at 37 °C in the absence of CB for various times, and then fixed and reacted with antisera against HA and β_2 -m. After 10 min the number of "uropod"-carrying cells with redistributed surface antigens had decreased from 40 to about 10%. After a further 50 min antigens were again homogeneously distributed.

Tables 1*b* and 3 show the influence of antibody plus colchicine on CB-induced redistribution of cell surface antigens. Measles-virus infected and uninfected Lu 106 cells incubated in the presence of 0.5×10^{-2} M colchicine and CB at $10 \mu\text{g ml}^{-1}$ showed "capping" of β_2 -m in 3% of all cells whereas 15% exposed to CB alone showed "capping". The number of cells exhibiting CB-induced polar distribution of measles virus HA was decreased in the presence of 10^{-2} - 10^{-3} M colchicine (Table 3). Colchicine, however, did not inhibit the formation of blebs. In its presence they were distributed all over the surface membrane (Fig. 1, Table 1*a*). So colchicine seemed to interfere with the movement of blebs to the "uropod".

Simultaneous exposure of infected and uninfected Lu 106 cells to antibody and CB, respectively, increased the frequency, rate and degree of redistribution of surface antigens significantly compared with exposure to each alone

Table 2 Kinetics of redistribution of haemagglutinin (HA)

Treatment with cytochalasin B for 60 min ($\mu\text{g ml}^{-1}$)	A Cells with capped HA (%)	B Treatment with cytochalasin B at 10 $\mu\text{g ml}^{-1}$		C Cells staining with trypan blue after treatment with DMSO 0.1% Cytochalasin B 10 $\mu\text{g ml}^{-1}$		
		Time (min)	Cells with capped HA (%)	Time (min)	No treatment	
0	0	0	4 3	0	4.7 5.6	
1	0	10	13 15			
5	13	30	28 25	60	3.0 4.7	5.3 5.7
10	41	60	44 31			3.0 4.0
				120	3.0 5.7	6.0 7.3
100	41	120	41 43			3.0 5.0

At least 200 cells were counted when percentage of cells was calculated. In B, two individuals calculated one set of values separately (A. E. upper and K.-G. S. lower value). In C, duplicate tubes were set up and the counting performed by one person. The experiments were performed with carrier cells, trypsinised and resuspended in Eagle's spinner medium supplemented with 5% foetal calf serum and kept in 37 °C for 60 min before the beginning of the experiments. Cytochalasin B was dissolved in DMSO and 10 $\mu\text{g ml}^{-1}$ of CB corresponds to 0.1% DMSO. The cells in A and B were fixed with 4% PFA before IF staining with anti-HA serum and examination.

(Table 1b). Thus, at a time when antibody-induced redistribution alone had not yet occurred, in the presence of both CB and antibodies, all antigens studied exhibited pronounced redistribution. This synergistic effect seemed to be independent of which of the reagents, the antibody or CB, were added to the cells first.

Cocapping of surface antigens and microfilaments

The behaviour of submembranous microfilaments of actin in relationship to the redistribution of surface components was examined in uninfected Lu 106 cells. Cells were suspended in 0.034 M sodium citrate, smeared on glass, fixed in dry acetone at -20 °C for 15 min and stained with a human serum containing antibodies to actin.

Before CB treatment, or when treated with DMSO used to dissolve CB, the cells showed virtually continuous IF-staining of their margin and a diffuse staining of the cytoplasm when reacted with anti-actin serum (Fig. 2). In contrast, CB-treated cells exhibited a redistribution of actin to the uropod which was completely reversible within 30 min (Fig. 2). This indicated that the redistribution of actin, and of cell surface antigens described above, were related to each other. To study this further double IF-staining with separate fluorochromes for actin and cell surface antigens was performed. The microfilament system was detected with the human actin antiserum²⁰ and a rhodamine (TRITC)-conjugated sheep anti-human IgG, absorbed with rabbit serum. Cell-surface antigens (β_2 -microglobulin, species-specific antigens) on Lu 106 cells were detected using the appropriate rabbit antisera and a fluorescein (FITC)-conjugated sheep anti-rabbit IgG, absorbed with normal human serum. Before CB treatment, the cells showed continuous IF-staining of their margins with both anti-actin serum (rhodamine) and antisera to surface antigens (fluorescein). On CB-treated cells the rhodamine staining was localised in the cytoplasm corresponding to the "uropod" but in many cells the redistribution was incomplete. To document the results further we took advantage of the synergistic influence of CB and antibody on the redistribution of cell surface molecules. This demonstrated cocapping of surface antigens and actin to the "uropod" (Fig. 3).

The specificity of the anti-actin serum was checked by absorption with purified actin²⁰. This clearly decreased the number of cells that stained with rhodamine as well as the

IF-intensity of the cytoplasm. Anti-actin serum did not stain viable Lu 106 cells.

Mechanisms of cytoskeletal control

Our principal observations were the coincident reversible CB-induced redistribution of microfilaments and cell-surface elements into a bulge of the cytoplasm and the antagonistic and synergistic influence of colchicine and antibodies, respectively, on this process. These results strongly support: (1) the existence of a connection, direct or indirect, between cell surface elements, microfilaments and microtubules; (2) the concept that cytoskeletal structures influence the behaviour of molecules in the plane of the membrane^{15-17,21}; and (3) the concept that microfilaments and microtubules are responsible for the cellular control of membrane mobility and integrity.

It is uncertain, however, whether microfilaments and microtubules antagonise or synergise in provoking and/or preventing membrane mobility. Cytochalasins have been suggested to affect the microfilament system by either disruption¹² or induction of contraction²². Therefore, interpretation of the results in terms of the function of microfilaments and microtubules depends on which of these two effects is favoured. It seems unlikely that the observed effects of CB on the cell surface¹⁴ are responsible for the redistribution of cytoplasmic microfilaments, unless these were exposed on the cell surface. Also it seems unlikely that the redistribution of cell-surface elements, so well correlated to the redistribution of microfilaments, should be due solely to a surface effect of CB. It should be re-

Table 3 Effect of colchicine on CB-induced redistribution of measles virus haemagglutinin

Experiment*	Treatment	Capped cells (%)
1	CB 10 $\mu\text{g ml}^{-1}$	39
	CB 10 $\mu\text{g ml}^{-1}$ + colchicine 10 ⁻² M	2
	CB 10 $\mu\text{g ml}^{-1}$ + colchicine 10 ⁻³ M	5
	CB 10 $\mu\text{g ml}^{-1}$ + colchicine 10 ⁻⁴ M	9
2	CB 10 $\mu\text{g ml}^{-1}$	30
	CB 10 $\mu\text{g ml}^{-1}$ + colchicine 10 ⁻² M	4
	CB 10 $\mu\text{g ml}^{-1}$ + colchicine 10 ⁻³ M	10
	CB 10 $\mu\text{g ml}^{-1}$ + colchicine 10 ⁻⁴ M	7
3	CB 10 $\mu\text{g ml}^{-1}$ + colchicine 10 ⁻² M	0
	CB 10 $\mu\text{g ml}^{-1}$ + colchicine 10 ⁻³ M	6
	CB 10 $\mu\text{g ml}^{-1}$ + colchicine 10 ⁻⁴ M	9
	CB 10 $\mu\text{g ml}^{-1}$ + colchicine 10 ⁻⁵ M	14

*Experiment 2 was performed in the presence and experiments 1 and 3 in the absence of FCS.

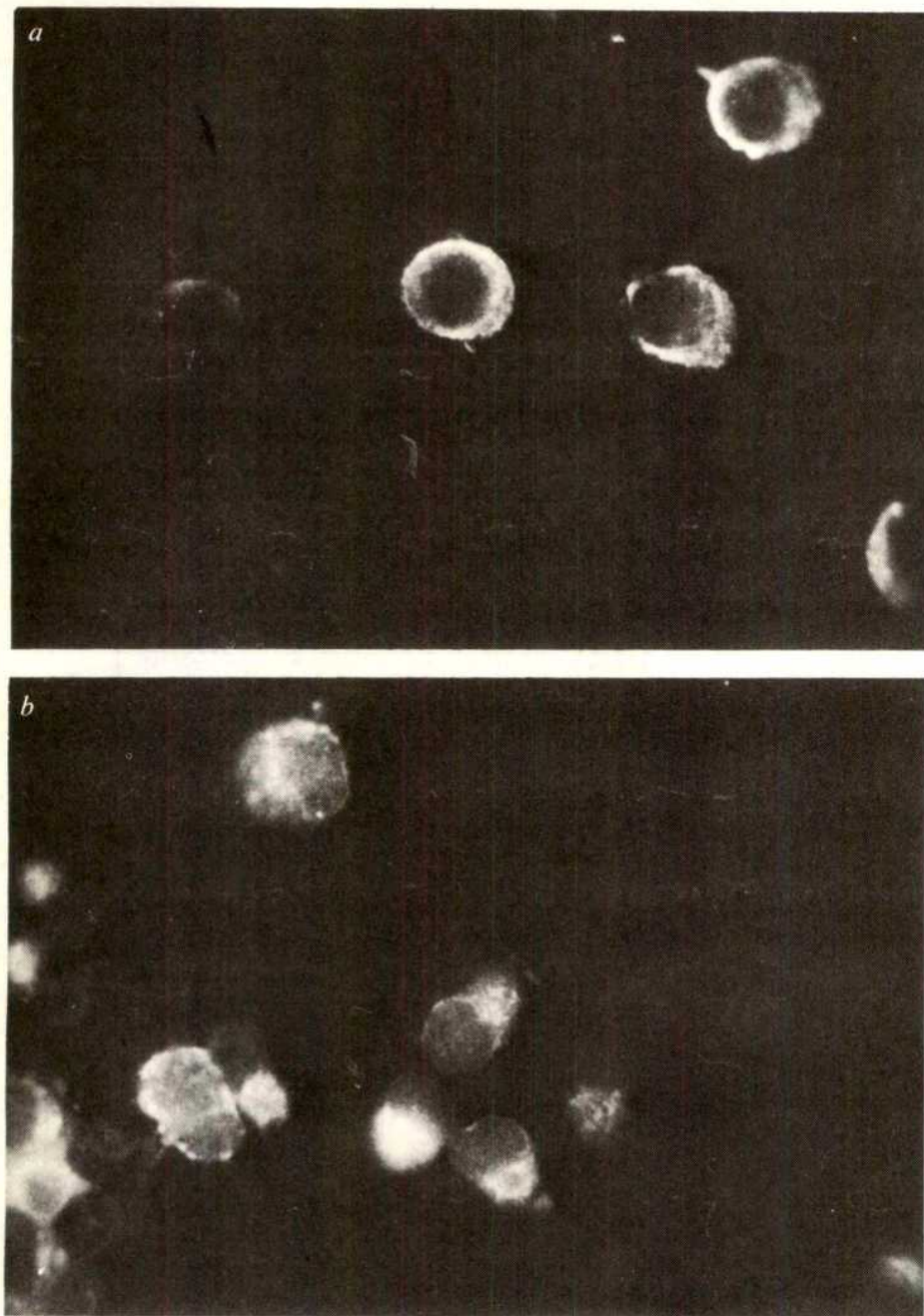


Fig. 2 Distribution of actin in uninfected Lu 106 cells *a*, before and *b*, after CB-treatment. Notice the redistribution of IF-staining to uropods.

membered that antibody-induced redistribution of cell surface antigens did not redistribute microfilaments in the absence of CB. Attempts to redistribute microfilaments by capping cell surface antigens in various test systems have failed (K.-G.S., unpublished observations). The reason for this may be that cross linking of cell surface antigens by antibody involves only microfilaments corresponding to the antigens studied, whereas CB redistributes more of the microfilaments, as the present data suggest.

It is interesting in the light of our result that microfilaments in some cell types contract after exposure to CB (ref. 22). Furthermore, cytochalasins induce the formation of knobby protuberances and the migration of these at the surface membrane^{23,24}. The protuberances resemble the blebs we obtained after treatment with CB.

The degree of redistribution varied for different antigenic specificities and cells as reported before with antibody-induced redistribution²⁵⁻²⁷. Measles virus HA exhibited the

greatest redistribution. It was present only on the capped area of the cell surface. HA was absent from the blebs whereas the other specificities were found along the margins of these protrusions. Thus, there may be a correlation between exclusion from blebs and a pronounced tendency to redistribution. This, together with the earlier findings^{7,25-27}, suggests that certain structures in the cell surface membrane, such as HA, are more dependent on the microfilament system than other membrane components.

Our results may seem difficult to reconcile with the observations that CB alone or with colchicine inhibits capping of lymphocytes¹¹ and that colchicine enhances capping in lymphocytes treated with con A (ref. 15). These discrepancies may be related to relative variations in the localisation, structure, quantity or association to membrane elements of microfilaments and microtubules in different cell types. This is supported by the absence of CB-induced redistribution in certain cell types in the study reported

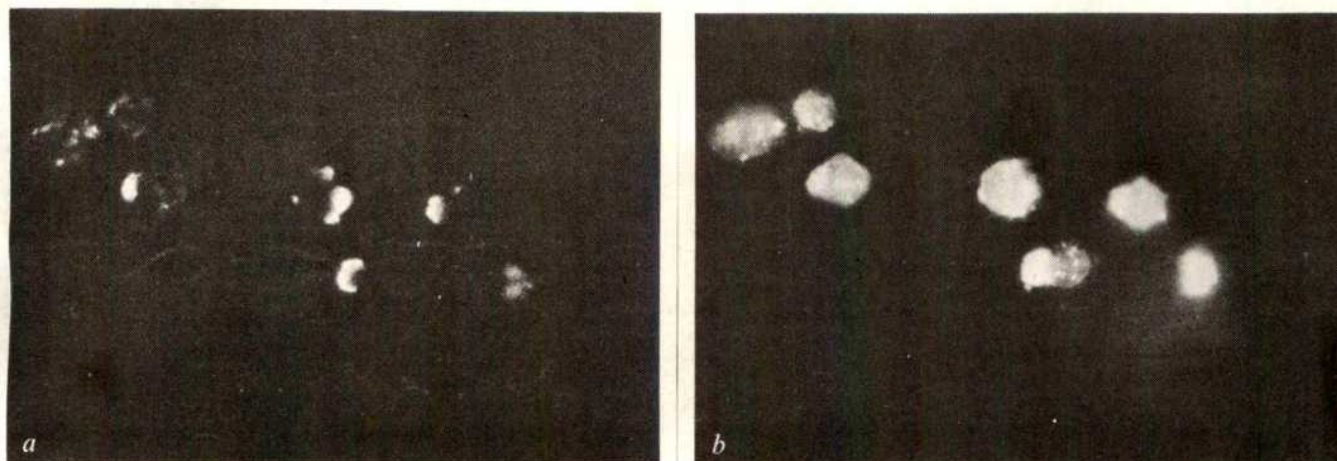


Fig. 3 Cocapping of β_2 -m and actin in CB-treated Lu 106 cells. *a*, Distribution of β_2 -m detected by indirect FITC-staining; *b*, Distribution of actin detected by indirect TRITC-staining. The live cells were first stained by rabbit anti- β_2 -m, diluted 1:20 and an absorbed sheep anti-rabbit IgG, diluted 1:5, 30 min, 0 °C respectively. Subsequently, CB 10 μ g in 1 ml was added and the cells incubated 60 min and then acetone-fixed. Actin was demonstrated by staining with a human anti-actin serum diluted 1:20 and an absorbed sheep anti-human conjugate diluted 1:2.5.

here. Such differences could conceivably influence both the nature and the magnitude of the cellular response to the two compounds. Thus, it is important to determine when CB and colchicine, respectively, induce and antagonise membrane mobility and to characterise the cellular basis for this difference between different cell types.

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X-ray diffraction studies of circular superhelical DNA at 300–10,000-Å resolution

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X-ray diffraction studies on circular superhelical DNA from bacteriophage PM2 at a very low scattering angle show that it is possible to measure the superhelical scattering function of the molecule. The results suggest that in addition to the primary supercoil, a higher order of supercoiling of the DNA is present, an effect which can be interpreted with a simple analogue.

THE supercoiling of circular double helical DNA, first demonstrated by Vinograd, has been studied by various physical and

chemical techniques¹. If the superhelix turns are fairly uniformly distributed, the periodicity should give rise to maxima in the X-ray diffraction pattern, the position of which depends on the precise geometry of the superhelix. Depending on the correct value of the superhelix density (which has been in dispute for some time) and on the addition of unwinding agents such as intercalating molecules (for example, ethidium bromide), the diffraction maxima are expected in the range from several thousand Angstroms down. Accordingly, we have explored the low angle diffraction from dilute solutions of circular superhelical DNA from PM2 bacteriophage² from about 10,000 Å down to about 300 Å. Two significant maxima are observed. The first, due to the "native" superhelix, appears at angles corresponding to a Bragg spacing of 337 Å. The second is observed at 2,100 Å, increasing with the addition of ethidium bromide (EB), in a manner to be expected if the latter unwinds

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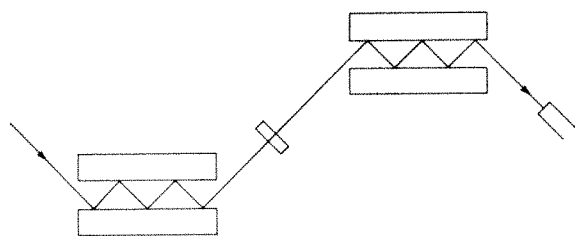


Fig. 1 Schematic drawing of the Bonse-Hart design for low angle X-ray scattering.

the helix. These results suggest that in addition to the primary supercoil, a higher order of supercoiling of the DNA is also present, an effect which can be interpreted with a simple analogue. A more general conclusion is that with the methods described here it is possible to observe the helix in an environment approaching that of *in vivo* conditions.

The DNA used in these studies was prepared according to the methods of Espejo *et al.*² from the bacteriophage PM2, which was grown and purified using modifications of previously published methods³⁻⁵. We verified that the intact DNA was in the closed circular supercoiled conformation by sedimentation velocity studies in alkaline and neutral CsCl and by Agarose gel electrophoresis. Nicked samples were prepared as required with pancreatic DNase.

The DNA usually had an absorbance ratio at 260–280 nm of 1.95–2.0. When examined in alkaline and neutral CsCl in the Beckman Model E ultracentrifuge using a Vinograd band-forming cell, only one band was visible. The DNA was further concentrated, when required, by vacuum evaporation. By this method, the solution could be reduced to about 1/100 of the original volume. After concentration, it was again dialysed against buffer (0.1 M NaCl, 0.2 M Tris (pH 7.5), 1 mM disodium EDTA), frozen quickly in a dry ice-acetone mix, and stored at -20°C . The DNA concentration used in the experiments was 2 mg ml^{-1} , at 0.1 M salt concentration.

Two sets of X-ray measurements were made. Those for scattering angles greater than $300\text{ s } 2\theta$ were taken with a Kratky camera using $\text{CuK}\alpha$ radiation; those in the scattering range

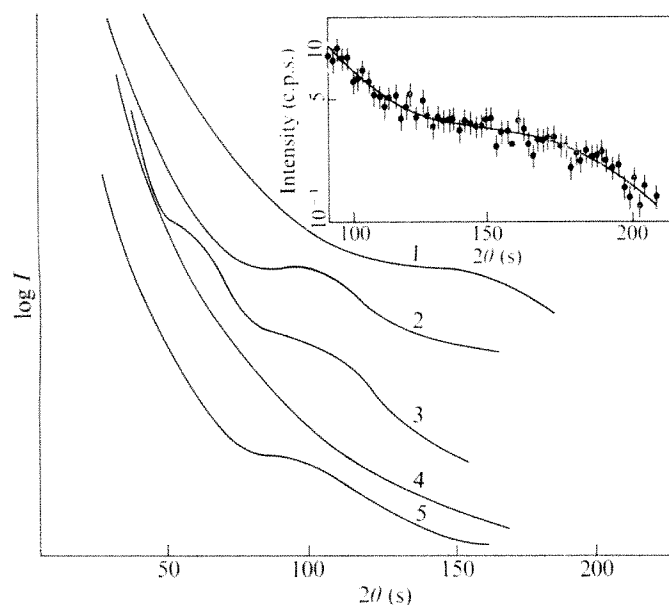
from $12\text{--}300\text{ s}$ were made with a small-angle apparatus constructed after the principle first demonstrated by Bonse and Hart⁶. The Kratky camera and its operation are well known⁴ and we need not discuss further the technique of that set of measurements. The Bonse-Hart apparatus is more unusual. In this arrangement two grooved germanium single crystals, one placed before and one after the sample, serve, by multiple reflection, to produce a narrow incident beam (in our case 12 s wide at half maximum). By this technique, highly collimated incident beams are produced without the necessity of using complicated slit geometries (Fig. 1). Since the rocking angle of these almost perfect crystals is so small (9 arc s) the analyser crystal and counter is placed only a few centimetres from the sample, instead of several metres, which would be required with a conventional low-angle camera. A resolution of $10,000\text{ \AA}$ is attainable, and the diffracted intensity at very low angles is much greater than with the Kratky camera. Because the beam is defined by the fixed reflection geometry, there is no way of increasing the intensity at the high angle end of the pattern; for the system studied here the intensity was at background level above $300\text{ s } 2\theta$, thus limiting the range of the apparatus to angles less than $300\text{ s } 2\theta$.

The zero position of the apparatus was determined by stepping through the main beam in steps of 1 s , with the beam attenuated to within measurable intensity by means of suitably calibrated copper filters. After the zero was located, the filters were removed and the patterns were recorded. Readings were taken between 12 and $300\text{ s } 2\theta$ with $\text{CuK}\alpha$ radiation ($\lambda = 1.54\text{ \AA}$). The sample was contained in a cell with a 1-mm path length with windows made from cleaved mica sheets, and the intensity difference between the cell full and empty after corrections for absorption varied from 30% at the lower end of the angular range angles to 15% at the higher end.

Table 1 Distances corresponding to peak maxima

EB/nucleotide	d_{Bragg}
0 (nicked)	
0 (un-nicked)	2,100
1:20	3,355
1:10	5,250
1:3	3,035

Fig. 2 Diffracted intensity (log scale) plotted against diffraction angle (2θ) in arc s. Curves: 1, Un-nicked PM2 DNA; 2, as 1 with EB added in 1:20 EB/nucleotide ratio; 3, as 1 with 1:10 EB/nucleotide; 4, nicked PM2 DNA; 5, as 1 with 1:3 EB/nucleotide. Inset, plot of a portion of curve 1, with error bars.



The scattering curves, corrected for background, are shown in Figs 2 and 4. We consider first the curves of Fig. 2, which show the scattering at very small angles. The molar concentration ratios of EB nucleotide are listed in the legend. A distinct, well defined peak is evident in curve 1 for supercoiled DNA without added EB. As EB is added, this peak moves in to lower angle, indicating an increasing correlation distance associated with the peak. In curve 3 the second-order peak is also evident, since the primary peak position (corresponding to a distance of $5,250\text{ \AA}$) occurs at such a small angle that the secondary peak is within the detectable range of the instrument.

Curve 4 is for a nicked sample where the superhelix is completely unwound. The scattering curve is smooth; the peak has disappeared. It reappears again in curve 5, at which concentration of EB the completely unwound superhelix begins to coil up in the opposite sense¹². The values of the distances corresponding to the peak maxima are listed in Table 1.

Figure 3 shows the reciprocals of the Bragg distances calculated from the peak maxima as a function of EB concentration. The relationship between the two is reasonably linear, except for the point on the left which is too high. This is in agreement with Freifelder's⁸ observation that the stiffness of the superhelix increases with addition of EB. Thus, Figs 2 and 3 are consistent with and most directly explained by ascribing the source of the scattering to a periodicity of the superhelix itself.

The peaks and the way they vary cannot be ascribed to maxima of a scattering function characterising the size and

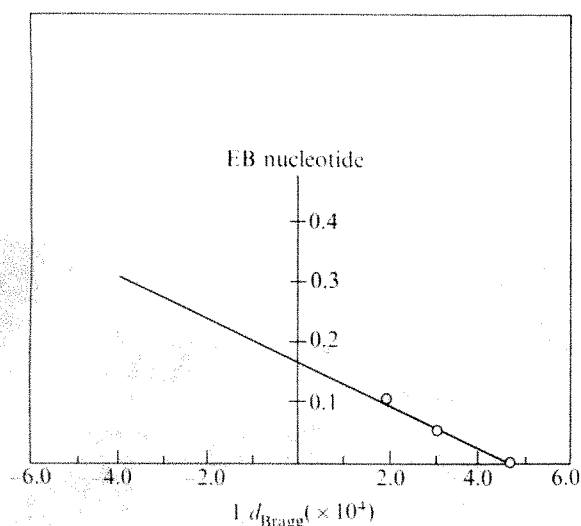
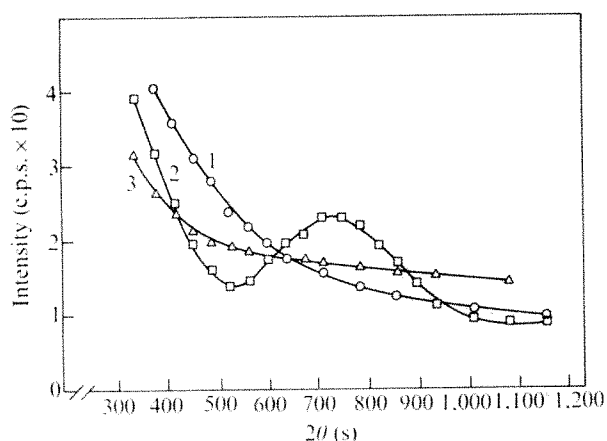


Fig. 3 EB/nucleotide ratio plotted against the reciprocal of the peak spacings; this latter is proportional to the rotation angle of the helix (see discussion in text). The 1:3 point is plotted as negative to show that the winding up is in the opposite sense.

shape of the DNA molecule as a whole. As examples, the scattering functions for loose spherical aggregates or elongated rod-like cylinders or ellipses are not consistent with the measurements. In the first case, a sphere radius derived from the position of the first maximum would result in a sphere volume so large that only a fraction (10^{-4}) of it could be filled by the superhelix volume. The length of the PM2 DNA molecule is 36,000 Å. For cylinders with radii of gyration consistent with the inner slopes of the curves, the peaks would have to appear at angles much further out on the scattering curve. Also, in this case, since the added EB causes the peak to move inward, consistent with an unwinding of the helix, its behaviour is opposite to that to be expected for the increase in asymmetry of an elongating rod-like structure, which would, of course, cause an outward shift of the peak. Finally, a random loop of these dimensions would not give peaks of any measurable intensity. This is evidenced by the nicked sample which does not show these effects at all. If we take the scattering curve of the nicked sample as resulting from a scattering function characteristic of the morphology (see also Fig. 4), the scattering function of which the peaks are a part appears to be superimposed on it. It is not possible at present, however, to isolate the scattering function of the helix from the total curve. A proper separation would require a better knowledge of the morphology of the

Fig. 4 Diffracted intensity measured at larger angles. Curves: 1, PM2 DNA with 1:53 EB/nucleotide; 2, PM2 DNA; 3, calf thymus DNA.



molecule, but even more important, an added difficulty is the nicking effect of the radiation on the helix, which makes quantitative intensities extremely hard to measure. The conformation of superhelical DNA is very sensitive to X rays⁹ and one of our major concerns was whether the material would last long enough in the superhelical form for the experiment to be done. After a 6-h experiment, ultracentrifugal analysis of the specimen indicated that 65% of the material was in the superhelical form whereas almost 100% was when the experiment began. This degradation was tolerable because while it changed the peak intensities relative to the background, it did not shift the peaks bodily and the spacings calculated from the maxima are unaffected. Because of the degradation, the intensities of the different curves of Fig. 2 are not directly comparable with each other and do not measure in any way the relative amount of helical material as a function of added EB. Also, because of the uncertainty in peak-to-background ratios, it was not felt appropriate to slit desmear the curves.

Table 2 Values of radius, helix repeat and contour length

<i>a</i> $d_{\text{obs}} = 337 \text{ \AA}$			
	<i>r</i>	<i>p</i>	<i>c</i>
	85	838	994
	90	608	830
	95	513	787
	100	460	779
	125	357	863
<i>b</i> $d_{\text{obs}} = 2,100 \text{ \AA}$			
	510	8,639	9,214
	550	4,124	5,380
	600	3,097	4,879
	700	2,442	5,030
	800	2,188	3,482

Before analysing these results further, we turn to the measurements with the Kratky in the range between 300 and 1,200 s 2θ, shown in Fig. 4. In these experiments the three systems examined were PM2 superhelical DNA with EB added in the ratio of EB/nucleotide = 0.19, corresponding to that concentration where the curve of Fig. 3 intercepts the ordinate (the unwound helix), PM2 superhelical DNA, and calf thymus DNA, which is not superhelical. It can be seen that whereas curves 1 and 3 show no indication of a maximum, curve 2 for the superhelix does. Its intensity is of the same order of magnitude as those of Fig. 2. We thus identify the peak as arising from the superhelical structure. The Bragg spacing corresponding to the peak maximum is ~337 Å. For helical structures randomly oriented, without long range order, the first maximum observed would be the first layer line¹⁰. The reciprocal space radius of this maximum is related to the helix repeat distance and to the helix radius. Since the first maximum of the first order Bessel function occurs at an argument of 1.84, we have from the relationship $2\pi rR = 1.84$, that R for the first maximum is equal to $1.84/2\pi r$, where r is the helix radius. The theory relates the first maximum to the helical parameters through the relationship

$$\left(\frac{1.23}{d_{\text{obs}}}\right)^2 = \left(\frac{1.84}{2\pi r}\right)^2 + \left(\frac{1}{p}\right)^2 \quad (1)$$

where p is the helix repeat. The factor 1.23 in the numerator on the left hand side is introduced to account for the difference in spacing measured in a single randomly oriented helix from that of a crystalline arrangement of helices¹¹. Similarly, for one turn of a helix the contour length c given by

$$c^2 = 4\pi^2 r^2 + p^2 \quad (2)$$

Our measurements give only d_{obs} and we cannot evaluate r and p separately. We can, however, determine values of p for various values of r and these are listed in Table 2, together with

the contour lengths calculated from equation (2). The minimum value of r from equation (1) is 80.2 Å. The values of p in Table 2 lie in the range between 800 and 300 Å, depending on the radius. Comparative values are 600 Å, deduced from hydrodynamic measurements by Gray *et al.*^{12,13}, and 300 Å, calculated by Wang¹⁴. The values of the radii, consistent with our measurements, are considerably greater than the diameter of the double helix, indicating that the hydrated superhelix is considerably

increasing distance upward from the bottom of the model. No correspondence with the elastic and mechanical moduli of the DNA is claimed for the analogue, nor can it depict visually the intervening space between the two double helices; but it shows that if the superhelix conformation is indeed primarily the result of a torsionally strained primary helix, then it will of necessity be accompanied by a second order helix, and this is what our measurements imply.

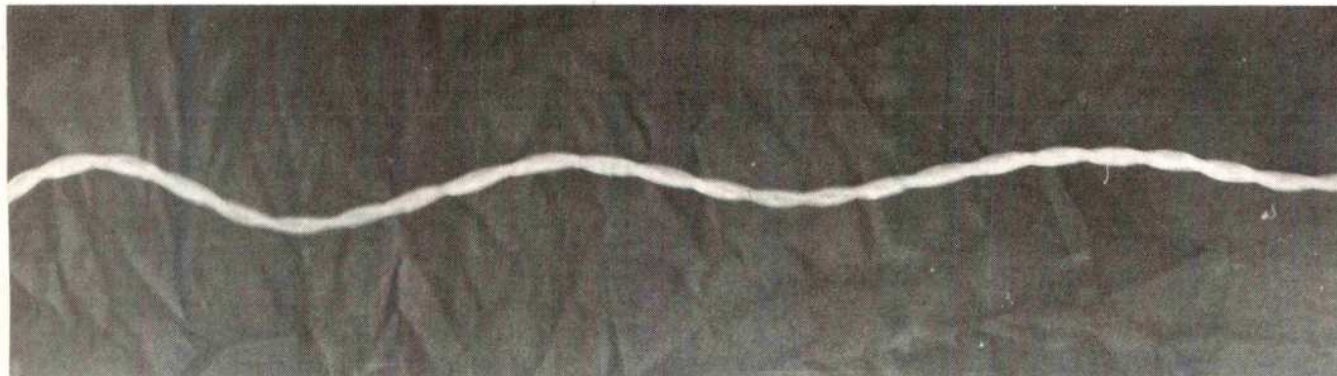


Fig. 5 Photograph of analogue model for the superhelix. The first-order superhelix is represented by the two intertwined chains which run from top to bottom of the model. The second-order superhelix is clearly seen as an additional superimposed helix (see text).

expanded, and should not be regarded as being equivalent to two DNA helical strands tightly wound together. Superhelical formation is the result of residual strain in the double helix, and does not result from attraction between the groups on its exterior; in fact, we would expect a net repulsion between peripheral PO_4^- groups. Thus we suspect that solvent interactions would make an important contribution to the final conformation.

These results place the interpretation of the curves of Fig. 2 on a firmer basis. We believe that they result from scattering from a higher order superhelix which is imposed on the primary superhelix by the mechanical properties of the double helical DNA molecule, whose strain is not completely relieved by the formation of the superhelix. The residual strain is distributed uniformly over the superhelix and leads it to adopt, in turn, a helical conformation of the opposite sense to the superhelix. To illustrate this with a model poses the difficulty of how to approximate the conformation of a strand of DNA when, in addition to being distorted by the residual strain, it is affected by interaction with the solvent medium. The resulting complex local distortions thus produced must be correlated over distances equivalent to the superhelix dimensions, and in general molecular models are not flexible enough to act as useful guides in determining the final conformation of such a complicated molecule when in the liquid state. As a means of circumventing this difficulty and to illustrate how a flexible helical macromolecule under strain is constrained to adopt the configuration we have described, Fig. 5 shows an analogue model. The analogue is made of rubber tubing which is twisted a few times, after which the two ends are joined to form a closed loop. The closed loop is the analogue of the double helical molecule and the imposed twist is the equivalent of the residual strain of the double helical configuration of the DNA. Because of the flexibility of the tubing, the strain is propagated uniformly along its length. When the tubing is released after closing, the loop supercoils. In the photograph the supercoil is identified as the series of small loops which extend from top to bottom of the model. Its period gives rise to the diffraction maxima observed at the higher angles. Its sense is opposite to that imposed on the tubing. Clearly evident in the photograph is another higher order helix of sense opposite to that of the superhelix. The analogue is of course distorted by gravitation, the effect of which can be clearly seen in the photograph, manifested by an increased pitch and decreased radius with

No theoretical analysis of the strain phenomenon exists, so there is no way to estimate how the strain is partitioned between the primary and second order helix, and thus be able to calculate the ratio of the number of turns between the two. We content ourselves with again listing in Table 2b the values of p , c and r corresponding to $d_{\text{obs}} = 2,100$ Å. The minimum radius is ~ 500 Å. For intermediate ratios of pitch to radius, we see from the contour length that the number of superhelix turns in one second order turn is about 6. Furthermore, since the superhelix length is 16,000 Å, there are three second-order turns per molecule.

Because of the nicking effect of the radiation and the low intensities involved, it is not possible to construct a complete composite curve from the two sets of intensity data. The overlap region of the scattering curves is differently affected by the different optical characteristics of the two diffractometers, and as we have noted, it is not possible, because of the nicking, to correct for this. Future work will be directed towards obtaining higher intensities, either by the use of a rotating anode tube, or by incorporation into the DNA of strongly scattering atoms. It will then be possible to extend the angular range of the Bonse-Hart to beyond 1,200 s. The nicking effect will be circumvented by using a position sensitive detector. It will then be possible to apply Fourier transform techniques to the data.

We thank R. Langridge and S. Lapedes for samples of nicked and un-nicked PM2 superhelical DNA and calf thymus DNA, and for stimulating discussions. We also thank D. Jones for help in preparing and standardising some of the samples in which EB was incorporated.

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letters to nature

Phase transition from baryon to quark matter

THE equation of state of matter compressed to supernuclear densities is of considerable interest in astrophysics. For example, pulsars are stars compressed to densities greater than the density of atomic nuclei and are thought to be composed primarily of neutrons, based on detailed calculations of the equation of state of baryonic matter assuming that interaction potentials for the baryons (nucleons and hyperons) are derived from low energy nuclear physics¹. There is, however, considerable evidence from high energy physics that baryons have structure, and it is currently believed that this structure is due to the fact that all hadrons consist of quarks bound by vector gluons². The fact that free, isolated quarks have not been observed has led to the speculation that quarks may be permanently bound inside hadrons³. Since the quarks inside the nucleon behave as free particles², the forces confining the quarks apparently become strong only when the distance between quarks exceeds the radius of a nucleon, $\sim 10^{-13}$ cm. When the density of matter is increased beyond the point that the mean distance between quarks in different baryons is $\ll 10^{-13}$ cm the description of matter as interacting baryons becomes invalid, and instead, a description of matter composed of quarks becomes relevant. At sufficiently high densities matter will behave like a relativistic gas of free quarks⁴ with $P \simeq \rho/3$, where P is the pressure and ρ is the energy density of matter. The density at which the baryon to quark transition occurs is of crucial importance to the structure of pulsars. General relativity implies that for a given equation of state there is a maximum energy density for stable stars¹. It is therefore of considerable interest to know whether the energy density of baryon matter at the baryon-quark phase transition is less than this maximum value. Indeed, it has recently been suggested⁵ that the existence of quark matter inside pulsars would allow larger masses for pulsars than was previously thought possible. We will show, however, that there are reasons to doubt this.

To determine the density for the baryon-quark matter transition one needs a theory of quark confinement. Although a detailed theory of confinement is not yet available, a phenomenological theory has recently been proposed⁶ in which the confinement is caused by a universal pressure B on the surface of any region containing quarks. Such a description is consistent with relativity and accounts for many observed

the density at which baryon matter changes to quark matter at zero temperature. At a fixed pressure P and zero temperature the appropriate phase of matter is the one which has the lowest Gibbs energy $\mu = (P + \rho)/n$, where n is the conserved baryon number density. Equating the Gibbs energy for quark matter and for baryon matter at the same pressure then determines the transition point.

Assuming that quark confinement is due to a phenomenological pressure B in the domain occupied by quarks, and that quarks moving inside this domain interact weakly by gluon exchange⁶, it is straightforward to calculate the thermodynamic functions of quark matter. The ground state of quark matter is a Fermi gas with all 'colour' states occupied for each level up to the Fermi level. When the Fermi energy of the quarks is large compared with the quark masses the energy density at zero temperature on dimensional grounds has the form

$$\rho = An^{4/3} + B \quad (1)$$

where A is a constant proportional to $\hbar c$, and n is conserved even when baryons no longer exist. The constant A depends on the dimensionless quark-gluon coupling constant g , and to second order in g we obtain

$$A = \frac{9}{4} \left(\frac{3\pi^2}{\kappa} \right)^{1/3} \left(1 + \frac{8g^2}{3\pi} \right) \hbar c \quad (2)$$

where κ is the number of quark 'flavours' contributing to the energy density. From equation (1) we obtain the pressure P of quark matter

$$P = \frac{A}{3} n^{4/3} - B \quad (3)$$

and correspondingly the Gibbs energy per unit baryon number, μ , as a function of P for zero temperature

$$\mu = 4 \left(\frac{A}{3} \right)^{3/4} (P + B)^{1/4} \quad (4)$$

Table 1 Baryon energy density (ρ_c) in units of 10^{15} g cm⁻³ where baryons begin to disappear

	$g^2 = 0.55$ $B = 9.5 \times 10^{34}$ erg cm ⁻³	$g^2 = 0.75$ $B = 5.1 \times 10^{34}$ erg cm ⁻³
Pandharipande-Smith (3)	2.7	4.0
Bethe-Johnson (I)	6.5	9.0
Bethe-Johnson (VH)	13	18

The values of g^2 and B are two sets of MIT 'bag' parameters which agree with observed hadron properties⁷, with $\kappa = 3$. Pandharipande-Smith and Bethe-Johnson refer to two recent models of baryon matter^{8,9}.

properties of hadrons when the effect of gluon exchanges between quarks is included⁷. We obtain here the equation of state of quark matter in this theory including gluon effects to first order in the gluon-quark coupling constant g . We then apply Gibbs' criterion for a phase transition to calculate

To find the baryon-quark phase transition we must equate μ as calculated from equation (4) with calculations of μ for baryon matter. Typical results of such a comparison are shown in Table 1. The results are given for the best currently available estimates⁷ of g and B and for two recent calculations^{8,9} of the properties of cold baryon matter. It can be seen that the transition takes place at baryon energy densities which are 10-60 times the baryon energy density in normal nuclei. Both the quark number density and energy density at the transition are higher than the corresponding baryonic densities indicating that this is a first-order transition. Since the baryon matter calculations were based on nucleon-nucleon potentials derived from low energy nuclear physics their validity at the transition density is open to question. Nevertheless, the calculations should be reliable in their indication that the transition takes

place at densities considerably in excess of those in normal nuclei. Furthermore, since the baryon-quark phase transition occurs at an energy density which is higher than the maximum density calculated for neutron stars^{9,10} speculations⁵ that the existence of quark matter inside pulsars would allow larger masses for these objects than had been calculated using baryon equations of state are unfounded.

Applying the equation of state for quark matter, equations (1-3), we now evaluate the maximum density P_m for stable quark stars in general relativity. This density is given approximately by the condition that $\gamma = \gamma_c$, where $\gamma = d \ln P / d \ln n$ is the adiabatic index for quark matter ($= \frac{1}{3}(1 + \rho/P)$ from (3)) and γ_c is a universal function of P/ρ (ref. 11). From this condition we find $\rho_m = 8.3B$ which is lower than the energy density, the baryon-quark phase transition for all the cases investigated here (see Table 1). Thus, a consistent application of the phenomenological MIT model for quark confinement to second order in g leads to the conclusion that stable quark stars at zero temperature do not exist.

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Note added in proof: after submitting our letter we received a preprint by G. Baym and S. Chin in which similar results for the baryon-quark phase transition are obtained.

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High electric fields from industrial stack plumes

THE use of electrostatic precipitators on an industrial stack to remove particulate matter and aerosol particles is now quite common. The plume emanating from such an installation is likely to be highly electrically charged, and consequently associated electrical effects should be observable downwind of the stack.

Measurements were carried out from July 6 to July 10, 1976 of the vertical component of the electric field downwind of a 110-m chimney stack of a cement works at Eastgate, Co. Durham, using a series of conventional field mills¹. Eastgate, ~33 km W of Durham City, is sited in a broad valley, 235 m above m.s.l., running E-W into the Pennine chain. The weather conditions were slightly unstable and cloudless with an easterly wind of between 3 and 4 m s⁻¹ measured at 2 m above ground. Averages of readings taken over 10-min periods at 15-s intervals yield electric field values in the region of +4,000 V m⁻¹ at sites within 500 m of the stack. Peak values up to +10,000 V m⁻¹ were recorded within the same distance from the stack. The direction of the electric field corresponds to a negatively charged plume caused by an applied -60 kV to the corona wires of the electrostatic precipitators. The high electric field values can be compared with a typical value of the normal fair weather electric field upwind of the stack of ~-200 V m⁻¹.

Large perturbations in the normal electric field were observable for distances up to 8 km downwind of the chimney stack. The decay in the electric field, E_p , at a position P was found to

agree with the fundamental line charge equation², modified by an empirical dissipation factor $\exp(-x/d)$ as follows

$$E_p = \frac{\lambda}{2\pi\epsilon_0 h} \left[1 + \frac{x}{(x^2 + h^2)^{1/2}} \right] \exp(-x/d) \quad (1)$$

where λ is the electric charge per unit length, h is the height of the plume, x is the distance of the position P downwind of the stack, ϵ_0 is the permittivity of free space, and d is the distance at which the electric field is reduced to $1/e$ of its initial value. Experimental results indicate that d possessed values of between 1 and 2 km. The effect of turbulent diffusion in the plume was estimated from the plume observations to be sufficiently small to validate the line charge approximation up to at least 2 km downwind of the stack.

In view of the high values of the electric field, a series of experiments was performed to establish the occurrence of point discharge. Measurements of point discharge current from an insulated elevated point, 9.25 m above ground level, were made at 15-s intervals by means of a Keithley electrometer, model 640, 700 m downwind of the stack. Simultaneous measurements were made of the electric field at a distance 10 m upwind of the point. It was found that an electric field of 2.2 kV m⁻¹ was necessary to initiate the point discharge. A maximum value of point discharge current of 3 μ A was observed over a 20-min period. It was also found that the point discharge current, i , and the electric field, E , followed the theoretical relationship³

$$i = k(E - E_0) \quad (2)$$

closely, where k is a constant, E is the value of electric field and E_0 is the onset value of electric field for the occurrence of point discharge.

In studying the dispersal of plumes by atmospheric turbulence, the electrical methods described here may provide a useful supplement to conventional concentration and dosage measurements^{4,5}. The flow of negative charge away from the stack in the form of the charged plume leads to an equal positive current flow down the stack to Earth. Taking into consideration the numerous chimney stacks in operation, it is reasonable to assert that this hitherto unknown charge separation process could significantly modify estimates of the Earth's electrical current 'balance sheet'⁶.

The presence of large electric fields in the vicinity of certain chimney stacks must result in significant point discharge currents occurring from sufficiently high metallic objects. If the objects concerned are poorly earthed they could acquire large electric potentials, which may well lead to electric spark production. This would not be of any practical consequence in most cases. If, however, the atmosphere downwind of the stack contained inflammable material, caused for example by an industrial leak, the occurrence of sparks could result in the ignition or detonation of the material.

The preliminary work described here will be elaborated in more detail in a subsequent publication.

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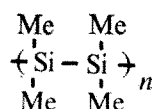
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Heat-resistant Fe–Cr alloy with polycarbosilane as binder

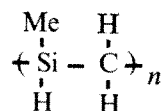
WITH the advances in recent years in high temperature gas-cooled reactors, jet engines and rockets, the constraints on heat-resistant materials are becoming more and more stringent; they must display high temperature strength, thermal shock resistance, high temperature corrosion resistance and toughness. Conventional heat-resistant materials can no longer meet these conditions¹. We report here the development of a new heat-resistant Fe–Cr alloy which has the required properties, based on the method for the conversion of an organic silicon polymer to an inorganic compound by heating which we have described previously^{2–3}.

Polycarbosilane (PC) is prepared as follows: suitable quantities of metallic sodium and xylene are mixed, and then heated to ~110 °C in a stream of inert gas. Dimethyldichlorosilane is then added. The chlorine in the dimethyldichlorosilane reacts with sodium on stirring, producing sodium chloride. By this reaction dimethyldichlorosilane turns into polysilane



which precipitates in the reaction vessel. The substance is recovered by filtration, washed and dried.

The purified polysilane is then put in an autoclave, and heated at 460 °C for 14 h with continuous stirring. The polysilane is thus converted to PC



with an average molecular weight of ~300–800. This is then heated to between 200 and 260 °C *in vacuo*, to remove the light molecular weight fraction by evaporation, and one is left with PC of average molecular weight ~1,000–2,000.

A mixture of powdered Fe–Cr alloy containing 13 per cent by weight of Cr (average grain size 3 μm) and PC of average molecular weight ~1,000 are weighed out in a ratio of 9:1. *n*-Hexane is added, the three are well mixed by kneading and the mixture is dried in air. At this stage, the particles of Fe–Cr alloy are uniformly covered with PC. It is hot pressed to obtain the moulding, using a carbon jig, at a pressure of 19.6 MPa in an atmosphere of Ar, with heating by high frequency induction, capable of giving 300 °C h⁻¹ and the moulding is kept at a maximum temperature of 1,100 °C for 30 min. The pressure is then released, the high frequency power source is switched off, and the moulding is left to cool naturally.

Powder X-ray diffraction using an Fe target with a Mn filter and transmission electron microscopic observation indicate the formation of spherical or ellipsoidal particle of Cr₇C₃ and CrSi₂ with a diameter of 1,500–2,500 Å. When polycarbosilane alone is heated at 1,000 °C in an inert gas

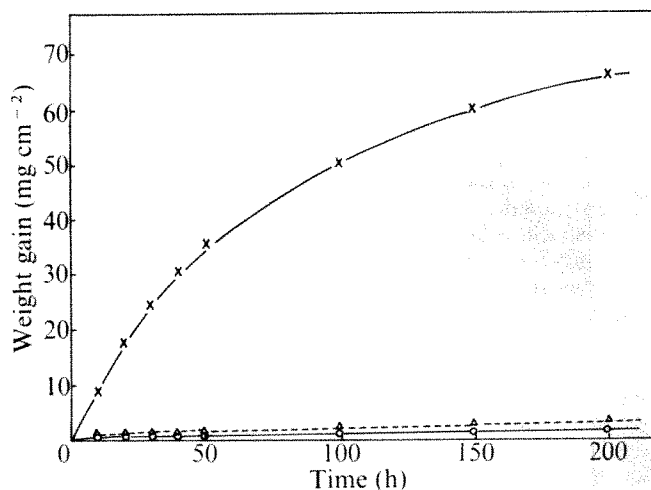


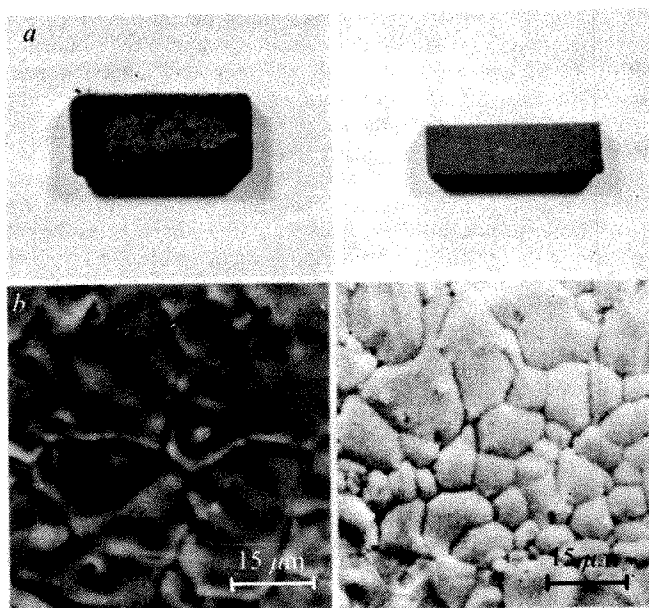
Fig. 1 The gain in weight against the heating time in air at 1,000 °C for the Fe–Cr alloy (x) and the Fe–Cr 10% by weight PC alloy (o); Δ, Ni–22Cr reference.

atmosphere, it decomposes, releasing CH₄ and H₂. The result is a skeleton of SiC, which is amorphous. If the Fe–Cr particles coated uniformly with PC are cold pressed and then heated at 1,100 °C, a reaction occurs between the Fe and Cr in the matrix and the amorphous SiC dispersed uniformly through the alloy, resulting in the formation of the silicide and carbide. A uniform distribution of silicide and carbide in the alloy is not obtained by introducing commercial α- or β-SiC into the powdered alloy and hot pressing.

Oxidation tests were made on the moulding obtained above, on a moulding made without PC and on the well know heat-resistant Ni–22Cr alloy as a reference, by heating in an electric furnace at 1,000 °C for 50 h. Figure 1 shows the weight gain from oxidation against the heating time for the Fe–Cr alloys at 1,000 °C in air. The weight increases in a parabolic curve in the Fe–Cr alloy without PC, but hardly increases at all in the moulding made with PC.

The surfaces of the mouldings after the tests were examined (Fig. 2a). Spalling of the oxidation film was found in the moulding without PC, but in the moulding with PC,

Fig. 2 a, Oxidation film of the alloy with (right) and without (left) PC after exposure to air for 50 h at 1,000 °C. b, Scanning electron microscopic observation of grain size in the two alloys (right with PC, left without).



only a slight film was observed which was firmly attached to the surface. This high resistance to oxidation may result from the stability of Cr_7C_3 and CrSi_2 against oxidation.

A value of 345 for the Vickers microhardness was obtained for the PC-coated alloy at room temperature—it is 100 in the PC-less alloy. Abrasion tests were made on the Fe–Cr alloys with Okoshi's testing apparatus. A disk of thickness 3 mm and diameter 30 mm was placed on the square $40 \times 40 \times 10$ -mm specimen, and a load of 7 kg was placed on the disk. The disk was rotated at 67 rad s^{-1} at room temperature. The value obtained for the abrasion resistance was $1.48 \times 10^{-6} \text{ mm}^2 \text{ kg}^{-1}$ in the Fe–Cr alloy with PC, and $7.10 \times 10^{-6} \text{ mm}^2 \text{ kg}^{-1}$ in the same alloy without PC.

The grain size of the mouldings heated at $1,000^\circ\text{C}$ for 5 h was examined with a scanning electron microscope after etching the surface with aqua-regia dissolved in glycerine. As seen in Fig. 2b, the grain size is $25 \mu\text{m}$ in the Fe–Cr alloy without PC, but in the alloy with 10% by weight of PC it is $9 \mu\text{m}$.

The large Vickers microhardness and the excellent abrasion resistance of the Fe–Cr alloy with PC can possibly be attributed to the existence of small particle Cr_7C_3 and CrSi_2 and the small grain size. This alloy may find many applications where heat resistance plus abrasion and oxidation resistance is required.

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SiC sintered bodies with three-dimensional polycarbosilane as binder

As is well known, silicon carbide is a practical heat-resisting material, because it has high strength and excellent oxidation, corrosion and thermal shock resistance. It is, however, difficult to obtain it as a sintered body because of its poor sintering characteristics. The usual method¹ for obtaining SiC with high flexural strength are to add a sintering promoter to fine SiC powder followed by hot pressing. The process is complicated and not suitable for obtaining large products. We here report the synthesis of high purity SiC mouldings with high mechanical strength by low temperature ($1,000$ – $1,400^\circ\text{C}$) sintering of SiC powders bond with three-dimensional polycarbosilanes. The method is based on the conversion of an organosilicon polymer to the inorganic compound by heat treatment^{2–5}.

The SiC powders used are 99.99% pure α -SiC, with an average particle size of $3 \mu\text{m}$. To these are added 10% by weight of polycarbosilane (PC)^{6,7} with an average molecular weight of 800 dissolved in a suitable quantity of *n*-hexane, which is then removed by evaporation. Particles $10 \times 30 \times 4 \text{ mm}$ in size were obtained by pressing at 196 MPa at room temperature. Their temperature was raised at 100°C h^{-1} to 700 – $1,400^\circ\text{C}$ in a flow of 200 ml min^{-1} of N_2 , and kept at the desired temperatures for 1 h. Sintered SiC particles were produced; there were no shrinkage or expansion.

The flexural strength of the SiC at room temperature was measured in three-point bending test with an Instron

bending tester under ASTM testing conditions. The $10 \times 30 \times 4$ -mm specimen was tested at strain rate $8.3 \times 10^{-3} \text{ s}^{-1}$ with the span of 20 mm. Figure 1 shows the bending strength against the temperature at which sintering occurred. The bending strength increases with temperature up to $1,100^\circ\text{C}$, beyond which it keeps constant at 61.7 MN m^{-2} .

To increase the strength still further, 'PC impregnation' was carried out. SiC particles obtained as above were heated together with PC powder in an autoclave under a vacuum at 100°C for 30 min, and then heated at 350°C for 1 h at a pressure of 9.8 MPa (of Ar). The particles were

Table 1 Characteristics of SiC with 10% by weight of polycarbosilane and also KT-SiC

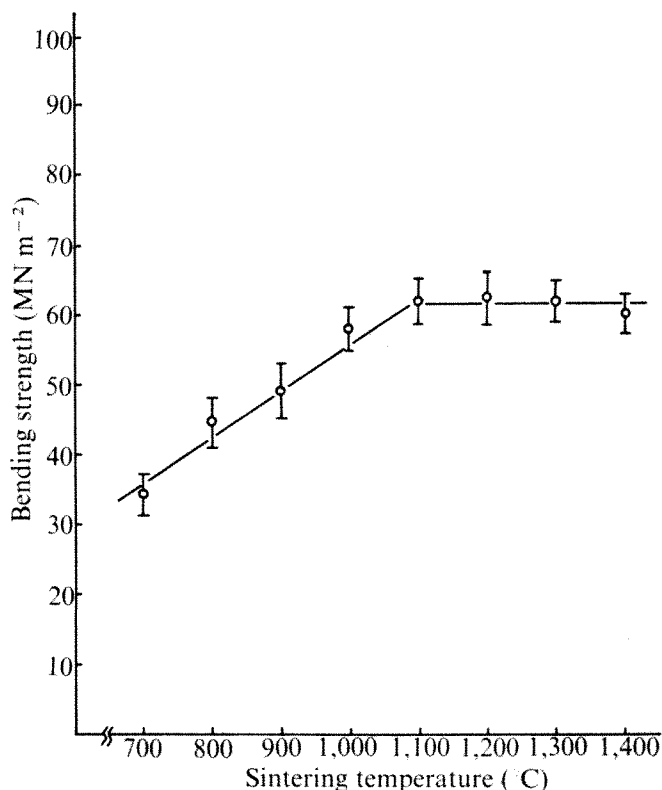
	SiC with PC	KT SiC ¹
Production temperature ($^\circ\text{C}$)	1,400	2,300 (hot press)
Impurity	$\sim 0\%$	Excess silicon or carbon
Apparent density (%)	70–80	100
Bending strength (MN m^{-2})	250	~ 168
Oxidation/acid resistance	Very high	Very high
Formability and dimensional precision	Excellent	Poor

then heated up to 700 – $1,400^\circ\text{C}$ at 100°C h^{-1} under a flow of N_2 . They were kept at these temperatures for 1 h.

The effect of PC impregnation is indicated in Fig. 2. It is seen that the apparent density and the bending strength both increase linearly with repetition of the process. The apparent density of the SiC particles after five cycles is 83.0% and the corresponding bending strength is 250 MN m^{-2} . A feature of the SiC particles thus fabricated is their exceptionally high bending strength for a low density. In Table 1, one sees that the bending strength of our SiC is 250 MN m^{-2} , while it is $\sim 168 \text{ MN m}^{-2}$ for KT-SiC.

The amount of PC added to the SiC was varied—and the addition of 10% by weight was found to be the most suitable. For a sintering temperature of $1,000^\circ\text{C}$ for example,

Fig. 1 Bending strength against sintering temperature.



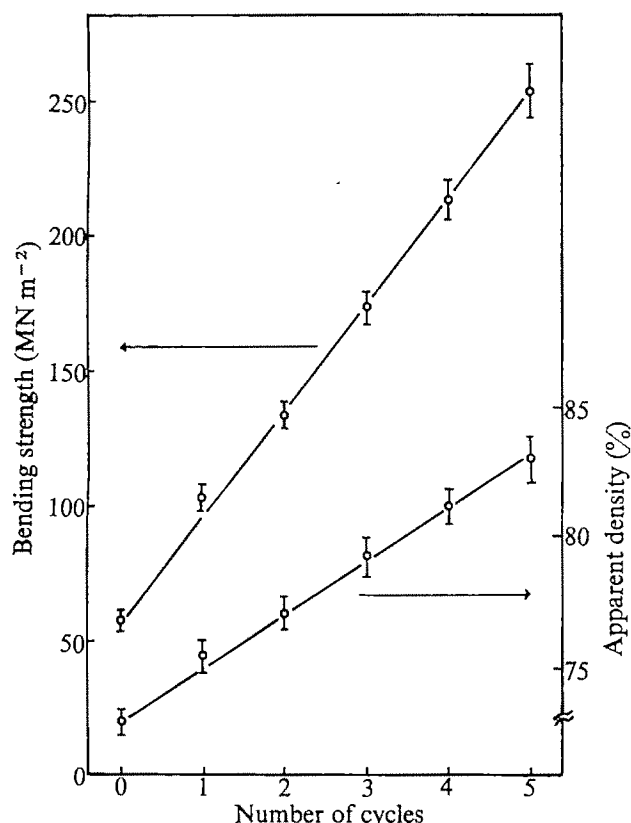


Fig. 2 Bending strength and apparent density against repetition of the PC impregnation cycle; sintering temperature 1,000 °C under flow of N₂, without heating in the air.

the bending strengths were 24.5 MN m⁻² for 5% by weight, 61.7 MN m⁻² for 10% by weight and 24.5 MN m⁻² for 15% by weight.

That SiC with PC has high mechanical strength, is interesting for the theory of sintering. In another experiment, SiC powder (particle size ~3 μm) and very fine SiC particles (~100 Å) (refs 2, 3) obtained by the decomposition of PC were sintered by heating. No contraction or expansion was observed. The diffusion coefficient of C in SiC is small— 3×10^{-13} cm² s⁻¹, at 1,800 °C, (S. Prochazka, unpublished)—so the mechanism cannot be simply explained by the normal idea of diffusional sintering. There is no dimensional change, and therefore one possibility may be condensation by PC evaporation.

When SiC particles were kept at 1,400 °C in air for 2, 5 and 10 h, the gains in weight from oxidation were 4.41, 5.70 and 7.01 mg cm⁻², respectively. Beyond 10 h, there is no increase. Bending strength after oxidation was measured. For sintering at 1,000 °C under N₂ flow, for example, it was 56.8 MN m⁻² in Fig. 1, but increased to 147 MN m⁻² after being kept in air at 1,400 °C for 10 h—the SiC is not only resistant to oxidation, but its bending strength increases. This phenomenon is being studied at present, but preliminary powder X-ray diffraction indicates that a very small amount of Si₃N₄O was produced on oxidation and this acts as a binder, thereby raising the bending strength.

The SiC was immersed in boiling solutions of 20% HCl and 50% HNO₃ for 100 h. It would have lost 0.05 mm of surface material in a year, and is therefore extremely stable in boiling acid solutions.

For SiC in current use, a different substance is used as the binder, so the SiC content in them cannot be >98%. For SiC with PC, however, there is hardly any chance for other elements than Si and C to be present. The product is thus of high purity; chemical analysis showed the SiC content to be almost 100%.

The SiC thus developed are easily produced industrially and may find a use in many fields, including the construction of chemical plants.

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Solar activity geomagnetic field and terrestrial weather

It has long been known that geomagnetic disturbances tend to recur after ~ 27 d (refs 1–4). More recently, this effect has been associated with the rotation of the interplanetary magnetic field^{5–8}, and some evidence for a relation between the interplanetary magnetic field and terrestrial weather has also been found^{9,10}. The interplanetary magnetic field is usually characterised by either two or four sectors, and this pattern rotates with a fairly well defined period of 27.1 ± 0.1 d (ref. 7). Such a periodicity leads naturally to the use of spectral analysis as an independent test of the reported association between the interplanetary magnetic field structure and terrestrial weather. We have obtained data on the geomagnetic activity index A_p for the 1964–70 from the National Oceanic and Atmospheric Administration and the vorticity area index used by Wilcox and his colleagues in their analysis⁹ for the same interval. We wish to form spectra of both time series and examine the spectra for common features which may be associated with solar related phenomena. Specifically we look for peaks in the power spectra of both time series with periods near 27.1 d.

Both the A_p and vorticity area index exhibit annual variations and long term trends. The annual variations and long term trends were removed from both data sets and the resultant series normalised so that the average of each index is zero. We have calculated least-squares estimates of the Fourier components of both normalised data sets for 1,921 periods (P) between 4 and 64 d. The estimates of the Fourier components a and b were calculated by varying a and b to minimise

$$V_3 = \sum_{n=1}^N \left[x_n - a \cos\left(\frac{2\pi}{P} n\right) - b \sin\left(\frac{2\pi}{P} n\right) \right]^2 \quad (1)$$

where the x_n are the normalised data points and N is the total number of data points.

After we had computed the Fourier components for both indices, we needed some way to compare the spectra and assess the significance of the results. We also needed some check that any periodicity found was indeed solar related, since both indices are of terrestrial origin. We have compared the spectra in three different ways.

One common method of assessing the significance of a least-squares fit¹¹ is to compare the residual deviation V_3 to the deviation of the data before fitting

$$V_1 = \sum_{n=1}^N x_n^2 \quad (2)$$

by forming the statistic

$$F_{2,N-3} = \frac{N-3}{2} \frac{V_1 - V_3}{V_3} \quad (3)$$

We have calculated the F statistic for each spectrum but, to compare the spectra, we tabulated the lesser of the two F statistics as a function of period. The largest peak in the resultant 'comparison spectrum' occurred at a period of 27.49 d. We may use the F statistic to determine the probabilities that the calculated reductions in variance are due to chance. Following Abramowitz and Stegun¹², we find the probability that the fit at 27.49 d arisen by chance for the A_p spectrum is $\sim 1.2 \times 10^{-4}$ and for the vorticity spectrum is $\sim 1.2 \times 10^{-6}$.

The F statistic calculated from the data is distributed only approximately as $F_{2,N-3}$, so that using such low probability estimates is probably not justified. We therefore arbitrarily use the lowest probability tabulated by Abramowitz and Stegun¹² for the F distribution which is 10^{-3} . If the A_p and vorticity indices are independent, then the probability that both indices would exhibit fits of this significance for a given period is $\sim 10^{-6}$. To obtain a significance estimate, it is essential to allow for the fact that the period was not chosen *a priori*, but inferred from the data. A generous estimate of the range of periods to be investigated is the range of observed synodic rotation periods of the photosphere of the Sun, namely 26.87–28.98 d (ref. 14). Accordingly, least-squares fits were calculated for 22 periods in this range. The probability of obtaining at least one of 22 probability estimates as small as 10^{-6} is $\lesssim 2.2 \times 10^{-5}$ if all of the periods are statistically independent. The length of our sample implies an intrinsic line width of 2.46×10^{-3} d⁻¹ and the spacing between the 22 periods is 0.77×10^{-3} d⁻¹. This indicates that not all of the 22 periods can be considered independent. We therefore think that calculating the probability estimates as if the 22 periods were independent produces a conservative probability estimate. Since for reasons stated above, we apply an arbitrary cutoff at 10^{-3} to estimates based on the F statistic, we have also calculated an empirical probability estimate. The F statistics for all the 1,921 periods analysed were ranked, and the probability of each fit estimated by the rank of the fit divided by the number of periods for which each spectrum was estimated. To compare the spectra, the larger of these two probabilities was tabulated as a function of period. There were two periods (19.88 and 44.52 d) with (chance) probabilities as low as or lower than the probability for the 27.49-d period. All three probabilities were, however, close together (26/1921, 27/1921, and 27/1921) and the next most significant peak had a probability estimate approximately twice as large (52/1921). If we combine the two empirical probability estimates in the same manner as the estimates from the F test, we find that the peak at 27.49 d has the smallest probability estimate ($\sim 3 \times 10^{-5}$) of any of the periods analysed by more than a factor of two. When we compensate for the fact that the period is chosen *a posteriori*, the empirical probability estimate that the observed result was produced by chance becomes $\lesssim 7 \times 10^{-4}$. Since both the A_p and vorticity indices are terrestrial, the assumption that the two indices are independent may not be justified. We therefore

explored a third procedure, based on the assumption that the relation between the A_p index and the interplanetary magnetic field structure is sufficiently well established that we may use the A_p spectrum to establish a rotation period. This assumption implicitly involves the supposition that there are no periodic meteorological phenomena with periods close to the solar rotation period that affect the geomagnetic activity index. We may then accept the probability estimates for the 27.49-d period in the vorticity spectrum. These estimates are $\lesssim 10^{-3}$ and $\sim 1.4 \times 10^{-3}$ for the F test and empirical probability estimates respectively.

Even if we accept the reality of the peaks in the spectra at 27.49 d, it is desirable to have some check that these peaks are associated with solar rotation. One fairly simple check is based on the fact that the Earth's orbit is slightly eccentric, and this eccentricity causes a variation of the apparent rotation period of the Sun through the calendar year¹⁵. The eccentricity of the Earth's orbit may easily be taken into account (to first order in the eccentricity) by slightly modifying the argument of the sine and cosine functions in equation (1). We have generated 'eccentricity corrected' spectra for both data sets and evaluated the results in the same manner as the uncorrected spectra. The F statistics for both A_p and vorticity indices were higher in the 'corrected spectra', but the probability estimates from the F test were not changed since we adopted an arbitrary cutoff at 10^{-3} . For the empirical probability estimates, the results were approximately the same as those for the uncorrected spectra; however, other peaks were degraded, with the result that the peak at 27.49 d had the lowest rather than the second lowest probability estimate. If we accept the period determined from A_p spectrum as a solar rotation period, the empirical estimate of the probability that the calculated fit in the vorticity index 'corrected' spectrum was produced by chance is $\lesssim 1.3 \times 10^{-3}$.

We have attempted to assess the significance of the observed peaks under various assumptions. The results vary from $> 99.99\%$ to $\sim 98\%$. We feel that the former estimate is unrealistically high; however, the decision to accept any of the estimates is subjective. Our own feeling is that the most reasonable procedure is to accept the period derived from the A_p spectrum as solar related. We therefore feel that the results of our analysis are significant at the 98% level. We do not feel that our analysis establishes a connection between the vorticity area index and solar activity, but we do feel that it provides supporting evidence for such a connection.

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Volatilisation from solid particles of the regolith

WE have previously¹ dwelt on the possible volatilisation of some elements from continuous melts of lunar surface material as a result of which it becomes depleted in elements such as Na, K and Rb, and so on. The mechanism of volatilisation proper was not confined to the liquid phase, and so they can also volatilise from the solid phase. Moreover, if solidification is not accompanied by crystallisation, the temperature dependence of the volatilisation time constant τ_v found in ref. 1 should remain unchanged.

Table 1 Calculated values τ_v for volatilisation of Na from lunar material (terrestrial time)

Particle size (cm)	Surface temperature (K)			
	1,500	900	500	300
1	15 h	5 d	25 d	300 yr
10^{-3}	0.15 h	1.2 h	0.25 h	3 yr

Table 1 gives the calculated values τ_v for the volatilisation of Na (see ref. 1) from the liquid and solid lunar material. For regolith particles the value τ_v is negligible compared with the geological time scale since $\tau_v \propto d$.

In ref. 1 the assumption that the lunar surface material was liquid was used only so that the complicated process of the transport of volatile elements to the surface could be ignored. In the solid phase, however, it is just their transport to the surface that may be the weak point of the calculation of the depletion process.

In this case the transport mechanism is mainly diffusive and, hence, a simple estimate of the diffusion time constant τ_d can be carried out

$$\tau_d = \frac{d^2}{D}; \quad D \sim \exp(-E_a/RT_s)$$

where D is the diffusion coefficient and E_a is the diffusion activation energy.

Since the dependence of τ_v on d is linear¹

$$\tau_v = \tau_{0v}d \quad (1)$$

where

$$\tau_{0v} \sim \exp[-(L-A)/RT_s]$$

and $(L-A)$ is the effective heat of evaporation^{1,2}, the depletion process will be limited by volatilisation ($\tau_v \gg \tau_d$) in the region of the smallest particles and by diffusion ($\tau_d \gg \tau_v$) in the region of large particles. The boundary between these regions $d = d_1$ can be determined from the condition $\tau_v = \tau_d$; then

$$d_1 = D\tau_{0v} \sim \exp[-(E_a - (L-A))/RT_s] \quad (1a)$$

The quantities E_a and $(L-A)$ have a similar physical meaning, and the difference between them $E_s = E_a - L + A$ should be close to the heat of adsorption, that is, it should be of the order of kcalorie mol⁻¹ (ref. 2).

Using equation (1), one can show that, at all temperatures of interest ($250 \text{ K} \lesssim T_0 \lesssim 1,500 \text{ K}$), the value of d_1 lies within the region of regolith particle sizes, $\sim 10^{-4}$ – 10^{-1} cm, and because $E_s > 0$ it increases with decreasing temperature.

In general, putting $\tau_0 = \max(\tau_v, \tau_d)$, one may assume that the rate of depletion of volatile elements from lunar material is determined by the time constant of the process 'weak point' τ_0 ($\tau_0 \lesssim 10^4$ yr for $d \lesssim 10^{-1}$ cm and $250 \text{ K} \lesssim T_s \lesssim 400 \text{ K}$). This interval corresponds approximately to the temperature

variations, T_s , of the upper layers of the regolith during the lunar day.

Since volatilisation and diffusion occur only in the day, and are almost totally switched off at night, the effective value τ_0 for the lunar day does not increase more than twice.

Next, it should be kept in mind that under meteoroid bombardment, and some other factors, the regolith particles, at least those from the uppermost layer ($h \simeq 10^{-1}$ cm), plunge into the lower ($h \lesssim 1$ cm) layers and then rise to the surface, intermixing at the rate $U_p \simeq 1 \text{ cm}/10^6 \text{ yr}$ (ref. 3). On the other hand, it is known^{3,4} that the rate of regolith formation is $U_f \simeq 10^{-1} \text{ cm}/10^6 \text{ yr}$.

Thus, for the regolith upper layer ($h \simeq 10^{-1}$ cm) one has the following inequality

$$\tau_0 \ll \tau_p \ll \tau_f \quad (2)$$

where $\tau_0 \lesssim 10^4$ yr, $\tau_p = h/U_p \simeq 10^6$ yr, and $\tau_f = h/U_f \simeq 10^6$ yr, τ_p and τ_f being the time constants of the corresponding processes.

The relationship between τ_p and τ_f shows that each regolith particle of a given layer was on the surface > 10 times whereas the first inequality in equation (2) implies that even a single sojourn of a particle on the surface is sufficient for the particle material to be significantly depleted in volatile elements.

Earlier we have shown theoretically and experimentally^{1,5} that the process of volatilisation from the liquid phase as a result of meteoroid impacts is inessential because the volume (size) of each melt is small, and the melts solidify very rapidly. These two factors also rule out the chemical fractionation of the lunar regolith by impact melting, proposed in ref. 6.

Meteoroid bombardment, however, can have a decisive effect on the process of volatilisation of Na, K and other elements from solid particles in the regolith, crushing and intermixing them. If this process really occurred (and occurs at present) on the Moon, then a chemical analysis of lunar rocks of the appropriate sizes could reveal the presence of a volatile-element concentration gradient.

Finally it should be noted that the approach we suggest may be also useful for solving some terrestrial ecological problems like that in ref. 7.

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Concentrations of dissolved copper in the eastern Atlantic Ocean 23°N to 47°N

CONSIDERABLE uncertainties still exist as to the abundances of most of the trace metals in seawater, including some of the more intensively investigated elements. Recent analyses of copper in surface waters south of New Zealand¹ and in waters from various depths over the East Pacific Rise² have given values of 0.06–0.40 $\mu\text{g l}^{-1}$, and a similar range has been found by M. J. McCartney (personal communication) for samples from the Mediterranean Sea and the north-eastern Atlantic Ocean. A geometric mean concentration of 0.26 $\mu\text{g l}^{-1}$ has been reported for samples collected off the shelf west of Scotland³. These values are either below

or at the lower end of the ranges reported for seawater by other workers. For example, extensive measurements⁴ of dissolved copper in surface waters have given average concentrations for various near-shore and open ocean regions which vary between 0.3 and 1.7 $\mu\text{g l}^{-1}$, with a mean of 0.8 $\mu\text{g l}^{-1}$, and a number of other recent investigations⁵⁻⁷ of North Atlantic Ocean waters also support an abundance value of $\sim 1 \mu\text{g l}^{-1}$. These contrasting findings may reflect real environmental variability but the alternative explanation that such variations are, at least in part, attributable to analytical problems, and especially to contamination, must also be considered. This communication reports values for dissolved copper in samples taken from the eastern Atlantic Ocean in October and November, 1975, which support findings of concentrations of $\sim 0.2 \mu\text{g l}^{-1}$. The results of measurements on these samples of the organically associated copper are summarised.

Surface samples were collected from RRS Discovery from a position forward of any discharges from the moving ship, using a plastic bucket with no metallic parts. Subsurface samples were collected using plastic water bottles. Metallic surfaces on the outside of these bottles, and the messengers employed, were coated with a plastic film. Samples collected from depths of < 500 m were filtered through Sartorius membrane filters of 0.45 μm average pore diameter, the filters having previously been leached with HCl. Samples from greater depths were not filtered since only a negligible amount of copper could be contributed from the small amounts of particulate material present. Dissolved copper was concentrated by uptake on Chelex-100 chelating ion-exchange resin after acidification of the sample (~ 800 ml) to pH 5, and the resin was eluted with 2 N nitric acid. The method was basically similar to that of Riley and Taylor⁸. This procedure leads to a measurement of the fraction of the dissolved copper which is available to the chelating resin. For the surface samples and a number of the subsurface samples from depths up to 1,000 m, analyses were also made on aliquots which had been photo-oxidised by ultraviolet irradiation for 5 h, using a 1,000-W medium pressure mercury arc lamp. The values obtained for the oxidised samples represent the total dissolved copper whereas the aliquots not treated in this way may contain some copper which is organically complexed in forms which are unavailable with this method of concentration. Further aliquots of most of these samples, also of ~ 800 ml, were extracted with chloroform at natural pH, without the addition of a complexing agent. The chloroform extracts were evaporated;

the residues were oxidised by evaporation with perchloric acid and redissolved in dilute nitric acid. Blank determinations were made for each procedure using only the reagents.

Concentration was commenced shortly after sample collection; the concentrates in dilute nitric acid were returned to the shore laboratory for determination of copper by atomic absorption spectrophotometry. Evaluation of the method has shown that the coefficient of variation of the procedure, as determined by analysing replicate samples at the level of 0.7 $\mu\text{g l}^{-1}$, is $\sim 4\%$; it gives essentially complete recovery of inorganic copper and the results agree closely with those obtained by solvent extraction of the complex formed with ammonium pyrrolidinedithiocarbamate. Blank values were 0.02 μg for the concentration procedure and $< 0.01 \mu\text{g}$ for the chloroform extraction.

The difference between the total and available dissolved copper fractions averaged $< 0.02 \mu\text{g l}^{-1}$ and so only the latter values are given in Table 1 with other chemical and hydrographic measurements; the samples were collected in sequence from north to south. The chloroform extractable fractions mostly ranged from undetectable to 0.02 $\mu\text{g Cu l}^{-1}$ with a single higher value of 0.04 $\mu\text{g l}^{-1}$. In coastal and estuarine waters, organically associated fractions of copper, operationally defined in ways similar to those used here, have been reported to be significant, ranging up to 50% or more of the total copper¹⁰⁻¹³. Our data indicate that in open ocean surface and intermediate waters, the fraction made available by oxidation of organic matter, and that which is chloroform extractable, each account for only $\sim 10\%$, or less, of the total copper. Measurements of operationally defined fractions do not enable any conclusions to be drawn as to the total extent of organic complexation. The difference in the magnitude of these fractions between coastal and oceanic waters, however, may reflect the different sources of organic complexing material in the near-shore and open ocean environments. The lower concentrations of such fractions in ocean waters, as found in this work, will require more sensitive analytical approaches for their fuller elucidation.

The concentrations of available dissolved copper ranged from 0.09 to 0.23 (mean 0.16) $\mu\text{g l}^{-1}$. This range agrees well with some other recent findings as discussed above but is substantially lower than the ranges reported hitherto for the North Atlantic Ocean⁴⁻⁷. For example, concentrations at various positions and depths in the eastern tropical region were found⁷ to range from 0.4 to 12.3 (mean 1.0) $\mu\text{g l}^{-1}$, while for a section from England to Barbados, extending

Table 1 Results for dissolved copper and hydrographic data

Position °N °W	Depth (m)	Salinity (‰)	Temperature (°C)	Dissolved copper ($\mu\text{g l}^{-1}$)	Dissolved silicon ($\mu\text{g atom l}^{-1}$)	Dissolved oxygen (ml l^{-1})
46°31' 09°36'	surface	35.575	—	0.22	—	—
44°52' 10°02'	surface	35.680	—	0.23	—	—
39°06' 10°32'	surface	36.013	18.3	0.22	—	—
38°05' 11°02'	surface	36.018	17.75	0.20	—	—
34°20' 12°40'	surface	—	21.7	0.18	—	—
31°11' 14°04'	surface	—	—	0.18	—	—
25°05' 16°24'	10	36.660	21.80	0.11	0.62	5.17
(Discovery Station 8932; bottom depth 2,560 m)	50	36.630	21.47	0.14	0.48	4.86
	100	36.588	19.12	0.11	0.62	5.92
	203	36.359	16.35	0.20	2.14	4.53
	353	35.946	13.89	0.13	4.69	3.96
	498	35.573	11.56	0.13	8.67	3.32
23°06' 17°51'	10	36.707	21.92	0.13	0.38	5.09
(Discovery Station 8933; bottom depth 2,985 m)	100	36.632	18.08	0.09	1.21	4.86
	431	35.636	11.94	0.10	7.74	3.70
	938	35.143	7.47	0.18	19.6	3.08
	990	35.190	7.04	0.12	19.9	3.34
	1,007	35.152	6.97	0.13	19.6	3.41
	1,513	35.177	5.50	0.23	22.1	4.15
	2,000	—	—	0.17	—	—
	2,176	35.034	3.78	0.21	28.3	5.16

into the eastern Caribbean Sea¹, surface concentrations of 0.47–1.8 (mean 0.93) $\mu\text{g l}^{-1}$ were found.

Our values for surface waters show a general decrease from north to south over the latitudes concerned. Variations in concentration with depth were not pronounced but suggest a slight depletion in the euphotic zone relative to the deeper water. A comparison of the trends in the concentrations of dissolved silicon and copper in the vertical profiles (Table 1) provides, however, no evidence that transference into deeper water by biological processes is a major influence on the distribution of dissolved copper.

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'Little Ice Age' palaeotemperatures from high altitude tree growth in S. Norway

THE sensitivity of tree growth to climate has led to reconstruction of past climates from long tree-growth records^{1,2}. Growth at the treeline in mountain regions and at the polar limits of tree growth is particularly useful because, in these locations, moisture is normally adequate and growth has been shown to be highly dependent on summer temperatures^{3,4}. Some other environmental factors have been recognised as influences on the growth of trees^{5,6}, but such factors are of relatively minor importance in this type of habitat. I reconstruct here a continuous record of summer temperature fluctuations representative of treeline conditions in the central southern Norwegian fjell since AD 1700. Combination of tree-ring data with information from glacier fluctuations over the same period shows short term oscillations of amplitude 1.0–3.0 °C superimposed on a long term increase in temperature of ~1.0 °C.

Two major limitations appear to restrict the use of tree-growth variations as palaeothermometers. First, even at the treeline, it is rare to be able to demonstrate a sufficiently high dependence of tree growth on summer temperatures. Secondly, calculation of standardised tree-growth indices (necessary to remove the effect of tree age on growth increment) results in the elimination of longer-term trends from the tree-growth record and hence the loss of information about longer-term temperature fluctuations. I show here that summer temperatures which are not subject to the above limitations can be reconstructed from tree growth. A Scots pine (*Pinus sylvestris*) tree-growth record, previously published by Slåstad⁷ from the treeline in upper Gudbrandsdalen (Fig. 1), and shown by him to be highly correlated with summer temperatures from Dombås meteorological station, is analysed for its palaeotemperature implications; independent glaciological and geomorphological evidence from the Storbreen glacier, Jotunheimen, is used to

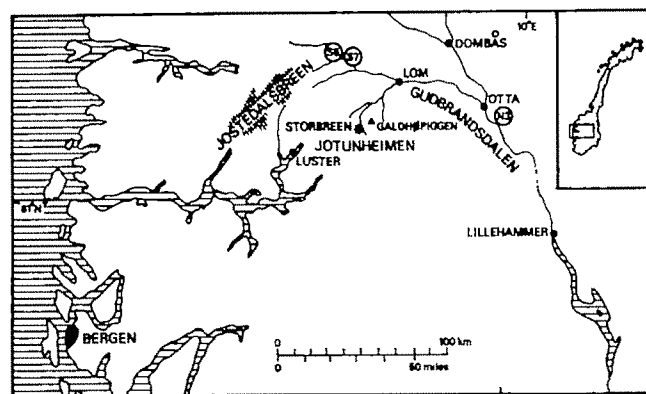


Fig. 1 Location of Slåstad's tree-growth sites—Skjåk 6 (S6), Skjåk 7 (S7) and Nord-Fron 3 (N3)—the Storbreen glacier and Dombås meteorological station.

adjust the record to take into account the long-term change in climate over the period of interest. Slåstad's tree-growth data are shown in unaltered form and after smoothing by harmonic analysis⁸ in Fig. 2.

Based on mean monthly 2 p.m. temperatures from Dombås, Slåstad found the strongest relationship to be between tree growth and an index of June and July temperatures, with July weighted twice, that is $(1 \times \text{June} + 2 \times \text{July})/3$. This relationship has been checked and is illustrated and given in mathematical form in Fig. 3 for the 50-yr period 1901–50 and for two 25-yr subperiods. The correlation coefficient (r) for 1901–50 is $+0.77$ ($p=0.001$); for 1901–25, $r = +0.84$ ($p = 0.001$); for 1926–51, $r = +0.79$ ($p = 0.001$). This relationship between tree growth and summer temperature is considered sufficiently strong to use regression of summer temperature index on tree-growth as part of the basis for reconstruction of temperatures before AD 1900. Before the relationship can be extrapolated into the past any long-term trend in tree growth and climate must be obtained from independent evidence.

Liestøl, working on the Storbreen glacier, has calculated the mass balance of the glacier since AD 1816 (ref. 9) and working on the glacier-foreland, I have reconstructed former glacier margin positions back to AD 1750. This information has been used to estimate the tree growth corresponding to the glacier in equilibrium (that is, no net change in the glacier mass budget and hence no net movement of the glacier margin) from AD 1700 to 1950. The resulting line corresponding to the equilibrium condition of the glacier (EE') is shown in Fig. 2b but full details of the methods used are given by J. A. Matthews, unpublished. Tree growth of less than the indicated critical value corresponds to a glacier advance; glacier retreat is indicated when tree growth exceeds the critical value. Fig. 2b shows that Storbreen was in equilibrium at a lower tree-growth value in the recent past than in earlier times.

The close agreement between glacier fluctuations and tree-growth variations is explicable in terms of similar summer temperature dependent models for tree growth at the tree line and for glacier behaviour^{4,12–14}. The altitude of the equilibrium line, the line dividing the glacier into accumulation and ablation zones, is a climate-sensitive parameter and is particularly influenced by summer temperature^{15,16}. Establishment of the altitudinal displacement of the glacier equilibrium line permits calculation of the temperature difference between the temperature necessary to maintain the present glacier in equilibrium and the temperature that was necessary in ~ AD 1750 (when Storbreen reached its 'Little Ice Age' maximum extent¹⁰). The altitude of the steady-state equilibrium line necessary to maintain Storbreen in equilibrium at present (1949–63) has been established as 1,690 m (ref. 9). Assuming a constant ratio between the accumulation and ablation areas on the glacier in the equilibrium condition, the AD 1750 steady-state equilibrium line was 65–70 m lower than the present (1949–63) steady-state

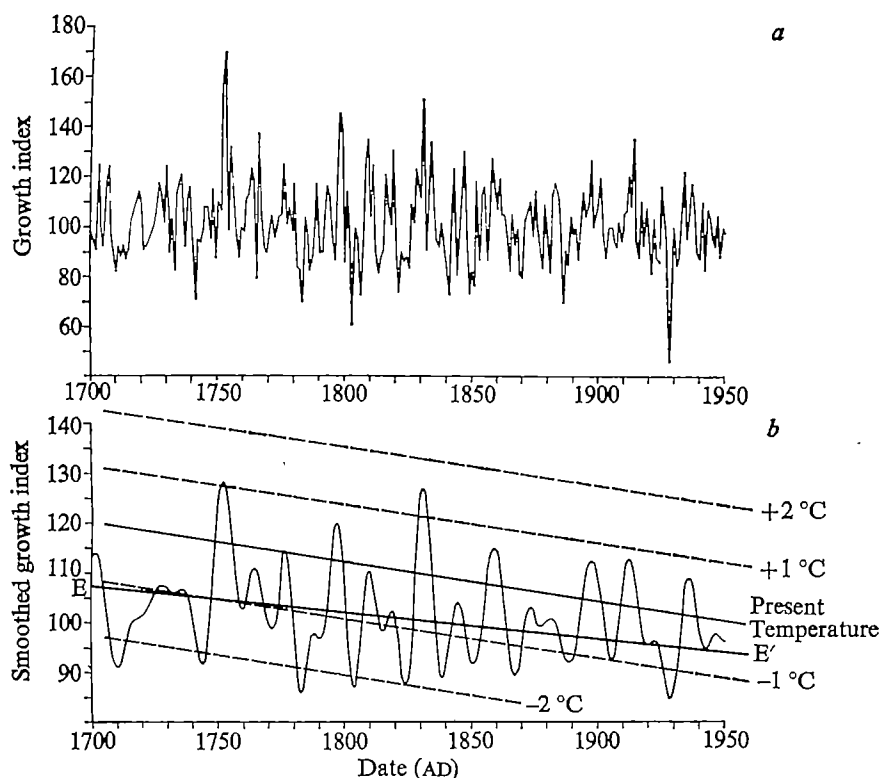
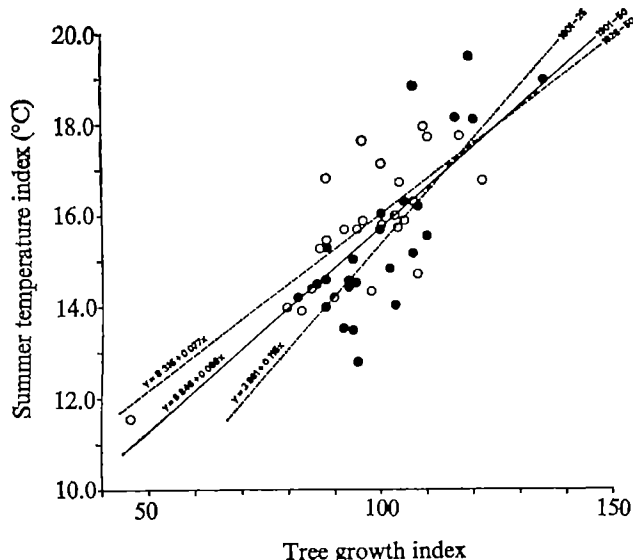


Fig. 2 Graphical representation of the variation in tree growth over the past 250 yr (a) and the smoothed curve after harmonic analysis (b). The smoothed curve is calibrated using an index of summer temperatures, $(1 \times \text{June} + 2 \times \text{July})/3$ and is expressed as differences from the present (1949-63). The line (EE'), which represents the tree-growth value corresponding to Storbreen in the equilibrium condition, was used in the calibration as explained in the text.

line (J. A. Matthews, unpublished). Thus the temperature necessary to maintain the glacier in equilibrium in AD 1750 was $\sim 0.47^\circ\text{C}$ lower than the temperature necessary to maintain equilibrium at present (assuming summer temperature dependence of the equilibrium-line altitude and a summer vertical temperature gradient of 0.7°C per 100 m). On average, between 1949 and 1963, the mean equilibrium line¹⁷ has been ~ 75 m higher than the steady-state line⁹. This means that a fall in temperature of $\sim 0.53^\circ\text{C}$ would be necessary for the present glacier to be in equilibrium and also that in AD 1750, when the glacier was in equilibrium, temperatures were $\sim 1.00^\circ\text{C}$ below present.

The information derived from equilibrium-line displacement permits calibration of the tree-growth variations in terms of summer temperatures. The calibrated curve (Fig. 2b) provides

Fig. 3 The relationship between the summer temperature index, $(1 \times \text{June} + 2 \times \text{July})/3$, and tree growth for the period 1901-50 (—). Relationships for the subperiods, 1901-25 (○) and 1926-50 (●), are also shown (---).



a continuous record of summer temperatures over the past 250 yr. At least 10 major oscillations are indicated, superimposed on a long-term temperature rise of 1.0°C since AD 1750. Cool fluctuations reached 1.0 – 2.0°C below present; warm fluctuations were up to 1.0°C above present temperatures. These temperature fluctuations are regarded as irregular rather than cyclic, although recent work based on the Grampian Mountains, eastern Scotland¹⁸ has suggested the existence of cyclic patterns in growth of Scots pine during the twentieth century.

A brief description of summer temperature changes, reconstructed from tree growth in 25-yr periods since AD 1700, is summarised in Table 1. The first half of the eighteenth century

Table 1 Summer temperatures reconstructed from tree growth during 25-year periods. The temperatures are based on the index $(1 \times \text{June} + 2 \times \text{July})/3$ and are expressed as differences from present (1949-63).

Date (AD)	Index ($^\circ\text{C}$)
1701-1725	-1.63
1726-1750	-1.14
1751-1775	-0.48
1776-1800	-0.87
1801-1825	-1.15
1826-1850	-0.46
1851-1875	-0.55
1876-1900	-0.43
1901-1925	-0.28
1926-1950	-0.34

is shown to have been at least 1.0°C cooler than the present, particularly early in the century when, from 1701-25, summer temperatures were on average 1.6°C below present. In contrast, the period 1751-75 was markedly warmer ($<0.5^\circ\text{C}$ below present) and corresponds well with the retreat of Storbreen and other southern Norwegian glaciers¹⁹ from their 'Little Ice Age' maxima reached \sim AD 1750. Renewed cooling occurred near the end of the eighteenth century (1776-1800) and continued into the nineteenth century (1801-25), but this cooling phase was not as great as that experienced at the beginning of the eighteenth century. Since 1825 no period has been $> 0.6^\circ\text{C}$ below present temperatures; 1825-1900 was from 0.4°C to 0.6°C cooler than at present and the twentieth century (1901-1950) is indicated as being warmer

than any previous 50- or 25-yr period with summer temperatures that were on average only 0.3 °C below those of 1949–63.

The temperature fluctuations that have been inferred from tree-growth variations are in broad agreement with and supplement existing knowledge from other sources such as instrumental records, stable isotope ratios in ice and wood, treeline fluctuations, glacier fluctuations, the extent of Arctic pack-ice, fluvio-glacial sedimentation rates, the incidence of rockfalls, avalanches and other natural disasters, vine harvest dates and crop failures^{19–25}. The continuous nature of the tree-growth record and the proximity of treelines to glaciers in many geographically separate localities should enable application of similar approaches in other areas and a contribution to be made to knowledge of recent climatic changes in time and space.

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Mass spectrometric studies of binding energies for nitrogen bases of nucleic acids *in vacuo*

FOR an understanding at the molecular level of the stability and interactions of nucleic acids, including the mechanisms of mutational events, the thermodynamic characteristics of base-pair formation must be determined¹. The most fundamental expression of these characteristics relates to the interaction *in vacuo*. We describe here the measurement of binding enthalpy of a single base pair from various combinations of bases *in vacuo*. We used pyrimidine bases methylated at the 1 position and purine bases methylated at the 9 position. As expected^{2,3}, the results obtained differ appreciably from those of earlier experiments⁴ on solutions, involving indirect methods of observing base pairing.

We have developed a direct method using mass spectrometers with a field ionisation source⁵. The field ionisation mass spectra contained peaks which could be attributed to ionised monomers and dimers only. The peak intensity was proportional to the concentration of the corresponding component near the field emitter. If concentrations are represented by $[M]$ for molecule and $[I]$ for ions then for the case of uracil and its dimer

$$[I_{uu}] = k_{uu}[M_{uu}]; [I_u] = k_u = [M_u]$$

where k_{uu} , k_u are ionisation efficiency factors.

k_{uu} = association constant =

$$\frac{[M_{uu}]}{[M_u]^2} = \frac{[I_{uu}]}{[I_u]^2} \frac{(k_u)^2}{k_{uu}} = \frac{[I_{uu}]}{[I_u]^2} k_1^*$$

k_1^* can vary from one experiment to another due to a change in the source geometry and emitter temperature.

In the temperature range studied, the temperature dependence of k_1^* is negligible. Figure 1 shows temperature dependence of the molecular ion peak intensity I^+ for cytosine, curves 1 and 2 based on electron impact and field ionisation measurements, respectively. The molecular beam was emitted from the orifice of a small Knudsen chamber (Fig. 1, ref. 5), its intensity being proportional to the saturated vapour pressure in the chamber. The heat of sublimation H_s is given⁶ by

$$\frac{d \ln(I^+T)}{d(1/T)} = \frac{-H_s}{R}$$

as 33.8 ± 0.5 kcalorie per mol by both measurements (curves 1 and 2, Fig. 1). On the other hand, the quartz resonator data which do not involve ions⁷ give a value calculated from

$$\frac{d \ln(v \times T^1)}{d(1/T)} = \frac{-H_s}{R}$$

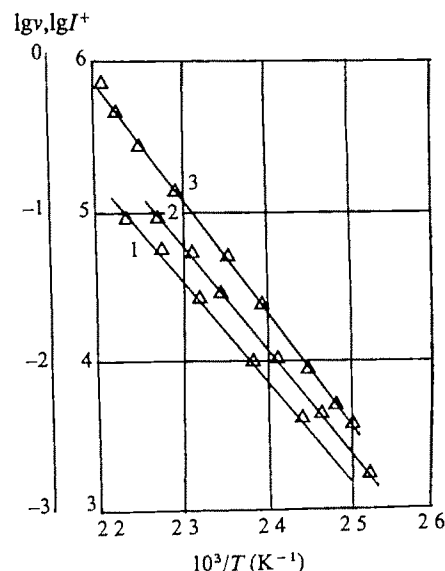
of 34.1 ± 0.3 kcalorie per mol. The agreement between all three values supports the validity of the treatment.

Thus the base-pair binding enthalpy, ΔH , can be found from the temperature dependence of the association constant

$$\frac{d \ln K}{d(1/T)} = \frac{\Delta H}{R} \quad (1)$$

It is, however, necessary to confirm that binding energy determined in this way corresponds to the specific hydrogen bonds responsible for the complementary interaction⁸. The field mass spectra of 1-methyl-U and of dimethyl-U and T are shown

Fig. 1 $\ln I^+(1/T)$ and $\ln v(1/T)$ for 1-methylcytosine derived from measurements by: 1, electron impact; 2, field ionisation; 3, quartz resonator method. v , Vapourisation velocity.



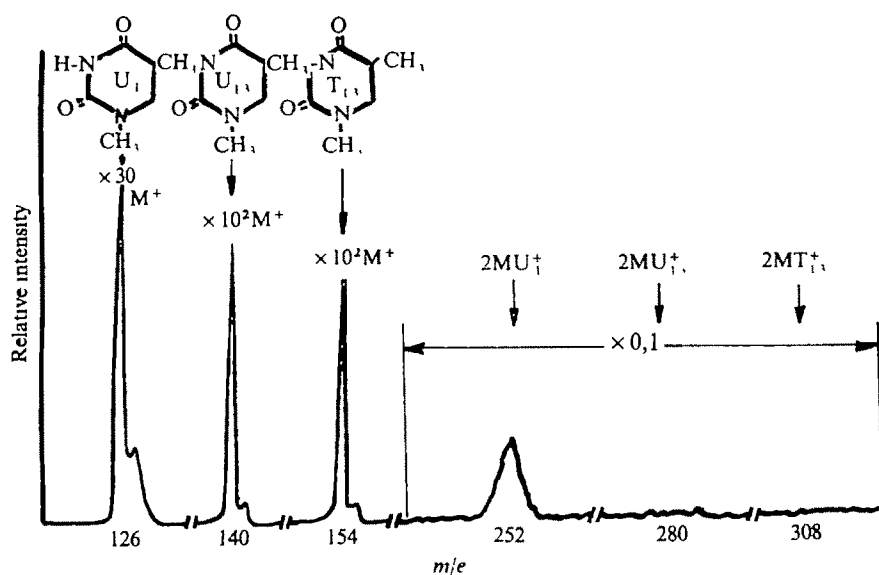


Fig. 2 Field ionisation mass spectra for 1-methyluracil (U_1); 1,3-dimethyluracil ($U_{1,3}$); 1,3-dimethylthymine ($T_{1,3}$). Evaporator temperature was 40 °C for the first and 30 °C for the second samples. Scale factor given for each peak.

in Fig. 2. In the latter two cases hydrogen bonds cannot be formed, and the peaks $2M^+$ are not observed in spite of the almost three times greater concentration of monomers at the emitter. The height of the $(M+1)^+$ peak for doubly methylated bases agrees well with the carbon isotope composition⁸, while for U_1 protonated ions were also observed, indicating that the proton of the 1-nitrogen atom participates in pairing.

The base-pair lifetime of 10^{-8} s (ref. 8) at $t = 25$ °C is much longer than the escape time of the ion from the ionisation zone (10^{-12} s) (ref. 9). Thus base pairing precedes ionisation and occurs between neutral molecules.

Table 1 Enthalpies of base-pair formation

Base pair	$-\Delta H^*$ (kcalorie mol ⁻¹)	$-\Delta H$ (kcalorie mol ⁻¹)		
		(2)	(3)	(4)
AU	14.5	7.21	—	6.2 ± 0.6
UU	9.5	5.42	—	4.3 ± 0.4
AT	13.0	7.0	7.7	—
TT	9.0	5.21	—	—
GC	21.0	16.79	21.4	$10 - 11.5$
CC	12.0	13.72	—	6.3

*The experimental error in ΔH is ± 1 kcalorie mol⁻¹.

The temperature dependence of the association constants in molecular beams of GC and AT is shown in Fig. 3. The curves for AU are similar. The binding energy is found from the slopes of the van't Hoff plots (Fig. 3). The temperature dependence of the relative association constant for the base pairs, observed in the same experiment, is particularly relevant because this is essentially independent of variable factors. This therefore gives a more reliable difference between binding energies for the base pairs in question than the absolute values. The difference so found is -8.5 kcalorie per mol for GC and CC, -5.5 kcalorie per mol for AU and UU and -5.0 kcalorie per mol for AT and TT.

The values of binding energies for various base-pair combinations are summarised in Table 1. The canonical GC pair can be seen to be more stable than the CC pair, while AU and AT are similarly stronger than UU and TT. When our binding energies are compared with theoretical predictions^{2,3}, the agreement is quite good for GC and CC pairs, while the experimental data for pairs containing A, U and T bases are a factor of 1.7 higher than predicted. Our binding energies for pairs *in vacuo* are about double those for bases interacting in chloroform⁴, in which, however, association is weaker than in the inert solvent carbon tetrachloride¹⁰.

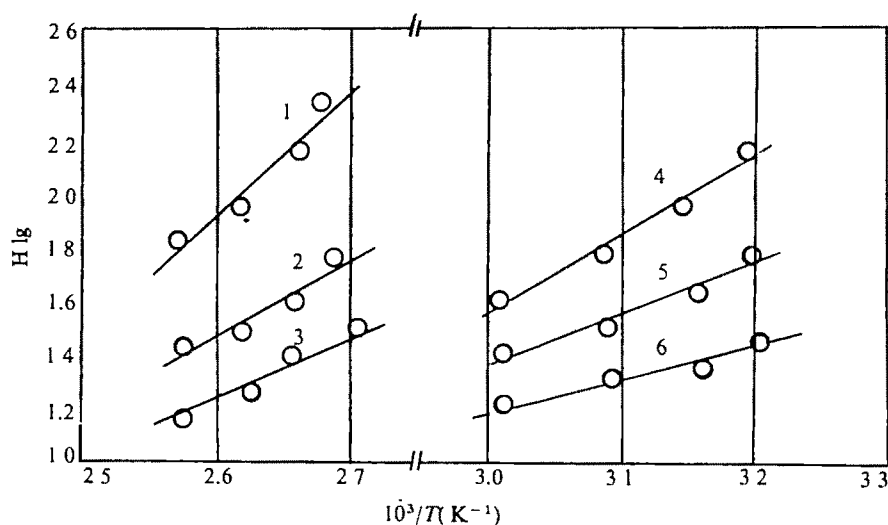
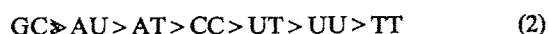
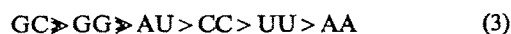


Fig. 3 van't Hoff plots for base-pairing associations: 1, GC; 2, CC; 3, GC-CC; 4, AT; 5, TT; 6, AT/TT.

The relative stabilities of base pairs determined previously⁶ are



This sequence agrees also with the results of dielectric measurement in non-polar solvents⁸, for example at 25 °C in benzene we have



(association constants 3×10^4 , 1.2×10^5 , 150, 28, 15, 8 mol⁻¹, respectively). It is interesting that the extrapolation of the plots in Fig. 3 down to 25 °C yields 10^3 and 40, which agree well with the relative association constants calculated from series (3). Thus our experimental method introduces no appreciable systematic errors.

Comparison of the experimental stability series (2) with theory shows good agreement⁸. The only exception is the CC pair, because according to theory, it should be more stable than the heteroassociates AU and AT. The discrepancies between the stability series (3), and the last column in Table 1, indicating that CC is at least of the same stability as AU and AT, suggest that the binding energy for CC requires further investigation.

Future experiments are expected to yield more accurate enthalpies of pairing of nitrogen bases *in vacuo* as well as data for other base-pair combinations which are important to an understanding of many problems concerning intermolecular and intramolecular interactions in nucleic acids.

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Interaction of heterocellular hereditary persistence of foetal haemoglobin with β thalassaemia and sickle cell anaemia

In normal adults a small amount of foetal haemoglobin (HbF) is detectable in up to 8% of the red cells (F cells)^{1,2}. In several inherited conditions, which include the Swiss³ and British⁴ types of hereditary persistence of foetal haemoglobin (HPFH), the proportion of F cells is increased in otherwise haematologically normal adults. It has been suggested (S. H. Boyer *et al.*, unpublished) that these conditions should be referred to as heterocellular HPFH to distinguish them from the more well known types of HPFH in which HbF is distributed throughout all the red cells (pancellular HPFH). Here we present evidence that the interaction of a gene for heterocellular HPFH with that for either β thalassaemia (β^{thal}) or sickle cell haemoglobin (HbS) results in the production of significantly greater amounts

of HbF than is usually found in β thalassaemia or sickle cell anaemia alone. The increased output of HbF which results from this interaction reduces the clinical severity of these common disorders. It seems that the genetic determinant for heterocellular HPFH is linked to the $\gamma\delta\beta$ -gene complex.

We have studied three families in which at least one member is a β^{thal} heterozygote with an unusually high level of HbF. The pedigrees of these families, together with the HbF, HbA₂ and F cell values of individual family members, are presented in Fig. 1. Detailed haematological data will be presented elsewhere (W.G.W. *et al.*, unpublished).

The proband of family M, of Italian origin, is a β^{thal} heterozygote with an atypically high HbF level. Her father has typical β^{thal} trait with the slight increase in HbF commonly found in that condition⁵; and both her mother

Table 1 Segregation of heterocellular HPFH and β^{thal} or β^{s} genes among the offspring of double heterozygotes

Family	β -chain marker	Offspring		ref.
		Parental type	Recombinants	
1	β^{thal}	2	0	11
2	β^{s}	2	1	12
3	β^{s}	7	0	13
4	β^{s}	3	0	14
S	β^{s}	8	1	This report
W	β^{thal}	5	1	This report
Total		27	3	

The excess of parental types indicates that the two genes are linked, with three recombinants (family 2, III.2, family S II.3, family W II.1) out of 30 informative offspring.

and brother, who are haematologically normal, also show a slightly increased HbF level and have elevated numbers of F cells of 12.9% and 12.1%, respectively. These latter individuals thus seem to be heterozygous for heterocellular HPFH; it is possible, therefore, that the high level of HbF observed in the proband results from the interaction of the gene for heterocellular HPFH with the β^{thal} gene.

The proband in family S, of Indian origin, has the haematological characteristics of sickle cell- β thalassaemia. Her haemoglobin consists of 68% HbS, 4% HbA, 2.9% HbA₂ and 24% HbF. The HbF is heterogeneously distributed among 73% of the red cells. This value for HbF greatly exceeds that normally found in sickle cell- β thalassaemia. Similar findings were observed in three siblings. The mother has typical β^{thal} trait, whereas the father has the sickle cell trait but also has an increased level of HbF and of F cells. It seems, therefore, that the proband and her three affected siblings have received the HbS and heterocellular HPFH genes from the father and the β^{thal} gene from the mother. The interaction of the three genes have produced a form of sickle cell- β thalassaemia with unusually high levels of HbF. None of these individuals has experienced the clinical symptoms usually associated with this disorder^{3,6}.

The proband of family W, of West Indian Negro origin, has the high HbF- β^{thal} trait, as does one of her children. Five other children have increased numbers of F cells, in four cases associated with increased levels of HbF. Her husband is normal.

In each of these families it seems, therefore, that a gene for heterocellular HPFH (probably identical to that previously described as Swiss HPFH, ref. 3) is segregating. It interacts with the β^{thal} gene to produce levels of HbF in the 6–15% range, and with the β^{thal} and HbS genes to produce HbF levels as high as 25%. How might this interaction result in such high levels of HbF? There is good evidence that, in patients with β thalassaemia or sickle cell anaemia, cells which contain HbF preferentially survive in

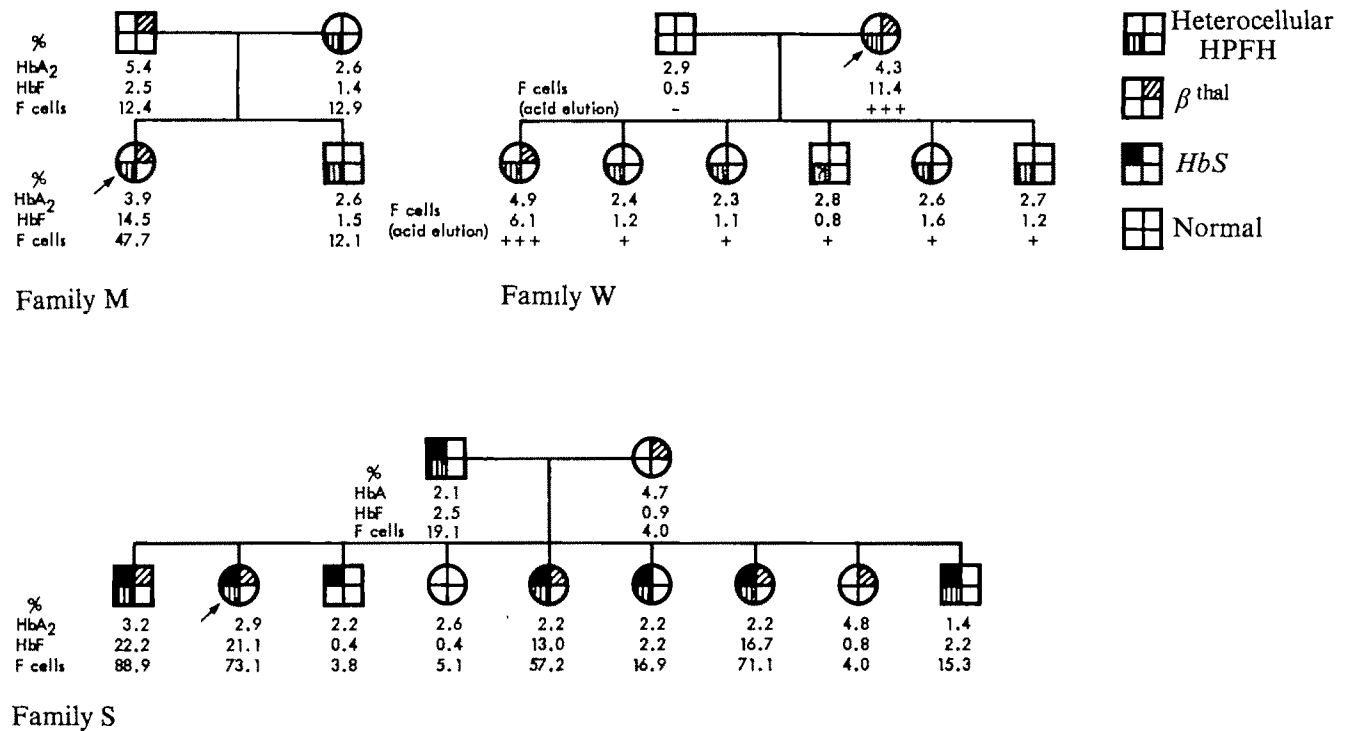


Fig. 1 Pedigrees of three new families in which the gene for heterocellular HPFH is segregating together with that for β thal or HbS. HbA₂ levels were determined by cellulose acetate electrophoresis⁶, the proportion of HbF was measured by alkali denaturation¹⁷ (upper limit of normal 0.9%), and F cells were measured by immunofluorescence⁸. For family W, semiquantitative estimates of F cell numbers were made in a separate laboratory, using the acid elution technique¹⁸. The genotype of individual II.4 in family W remains in doubt since although his HbF level fell within the normal range, he has more F cells than are found in normal individuals.

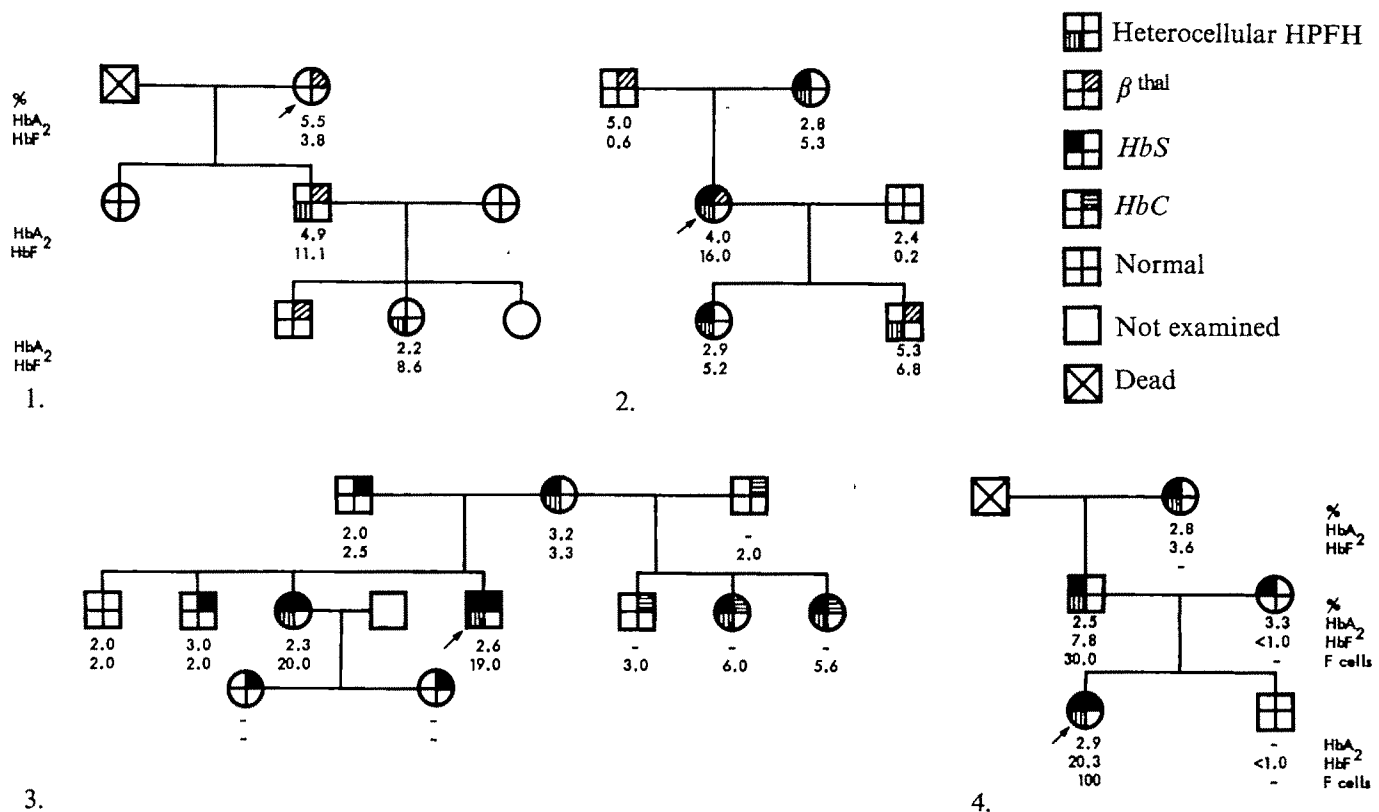


Fig. 2 Pedigrees of four families, previously reported¹¹⁻¹⁴, redrawn to show the interaction of heterocellular HPFH with HbS and/or β thal. The normal range for HbF in some of these reports may be as high as 2.5%. The distribution of HbF in the two HbS homozygotes in family 3 was described as homogeneous¹³. However, the difficulties involved in interpreting the results of the acid elution test¹⁸, and the fact that with 20% HbF most cells should contain HbF, suggest that the high HbF HbS homozygotes in this family can also be interpreted as due to the interaction of heterocellular HPFH with HbS.

the bone marrow or peripheral blood, respectively^{7,8}. It has been suggested that the elevated levels of HbF found in these conditions result from selective survival of the F cell population^{9,10}. If, therefore, in addition to the β^{thal} or HbS genes, there is a gene for heterocellular HPFH increasing the size of the F-cell pool on which the selective processes can act, a high level of HbF should occur. If this is the case, the level of HbF attained will depend on the degree of selection of the F-cell population and therefore on the severity of the condition. The finding of higher levels of HbF in the sickle cell- β thalassaemia heterozygotes than in the β^{thal} heterozygotes is fully in keeping with this prediction.

At least four other families in which it seems that interactions have occurred between the heterocellular HPFH gene and either the β^{thal} or the HbS genes, have been reported previously¹¹⁻¹⁴. Pedigrees of these families, modified according to this interpretation, are shown in Fig. 2. Genetic analysis of these families, together with those which make up the present report, indicate that the gene for heterocellular HPFH is inherited as an autosomal dominant. Furthermore, because the seven families have genes for both heterocellular HPFH and either β^{thal} or HbS segregating, it is possible to test for linkage of the gene for heterocellular HPFH to the β^{thal} or HbS genes. In this analysis we assume that the β^{thal} gene is very closely linked, if not allelic, with the β -structural gene since only one doubtful crossover has been observed in 62 offspring of informative matings⁸. The results, shown in Table 1, indicate that there is a large excess of parental types over recombinants among the offspring and provide good evidence that the gene for heterocellular HPFH is linked to the $\gamma\delta\beta$ -globin gene complex. The frequency of recombination between the gene for heterocellular HPFH and the β -structural gene, three out of 30, compares with no recombinants observed between the δ - and β -structural loci out of 63 offspring of informative matings¹⁵, and at least one, and possibly 3, recombinants between the β^{thal} and δ -structural loci out of 58 informative offspring¹⁶.

The action of the gene for heterocellular HPFH seems to increase the number of F cells rather than the amount of HbF per cell, since there is a linear relationship between the number of F cells and the level of HbF, both in normal adults and in those with heterocellular HPFH (W.G.W. *et al.*, unpublished). Although nothing is known of the mechanism by which this is brought about, it is intriguing that such a controller gene should be linked to the $\gamma\delta\beta$ -structural gene complex. In all these families the gene for heterocellular HPFH has been found *cis* to the β^{thal} gene and *trans* to the β^{thal} gene. We would predict that there should be a marked survival advantage in having the gene for heterocellular HPFH linked *cis* to the β^{thal} locus. The advantage which the β^{thal} gene has in malarious areas would then be maintained without loss of β^{thal} genes in the homozygous state since they would be protected by the high levels of HbF. The mild clinical condition of the HbS homozygotes^{13,14} and HbS- β^{thal} compound heterozygotes¹², who also carry the gene for heterocellular HPFH, confirm these predictions. It will be of great interest to study patients with sickle cell anaemia or homozygous β thalassaemia who have unusually mild courses and high levels of HbF, to determine whether they represent further examples of the interaction of the gene for heterocellular HPFH with these conditions. Such a project is already under way in Saudi Arabia where the Shiite Arab HbS homozygotes have a very mild sickling disorder due to the presence of 15-30% HbF (ref. 16).

The study of heterocellular HPFH is of considerable practical importance because it is clear that if it were possible to increase the F-cell population in patients with sickle cell anaemia or β thalassaemia, the clinical severity of these disorders would be reduced.

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Chromosome 21 does not code for an interferon receptor

It is well established that human cells which are trisomic for chromosome 21 are more sensitive to the antiviral activity of interferon than normal diploid cells¹⁻⁴. If chromosome 21 codes for an interferon receptor site localised at the cell surface, as suggested^{4,5}, one might expect cellular binding of interferon to increase in the order monosomic 21 cells, disomic 21 cells and trisomic 21 cells. This did not prove to be the case. All cell types bound interferon equally well. In view of these and previously⁶ reported data, it is postulated that chromosome 21, if it codes for a protein involved in the interferon response of the cells, does not specify the receptor molecule *per se* but another molecule that is involved in the processing of the antiviral message from the cell surface to the interior of the cell.

Human interferon, whether induced by viruses, double-stranded RNA or phytohaemagglutinin in either leukocytes, fibroblasts or lymphoblasts, seems to elicit a higher antiviral response in human skin fibroblasts which are trisomic for chromosome 21 (T-21) than in cells which are disomic for chromosome 21 (D-21); D-21 cells are in turn more sensitive to the antiviral action of interferon than cells which are monosomic for chromosome 21 (M-21)¹⁻⁶. To account for the increased interferon sensitivity of T-21 cells, different hypotheses have been proposed. According to Tan^{1,2}, chromosome 21 governs the synthesis of a secondary antiviral protein, whereas Chany⁴ postulated that chromosome 21 would carry the structural genes for an interferon receptor. From studies with interferon covalently bound to an insoluble matrix it was inferred that the interferon receptor site may be located at the outer cell membrane⁷⁻¹⁰. That such a receptor site could be specified by chromosome 21 was suggested by Revel *et al.*⁵. They found that an antiserum against a chromosome 21-directed cell-surface component reduced markedly the antiviral action of interferon in normal human diploid cells.

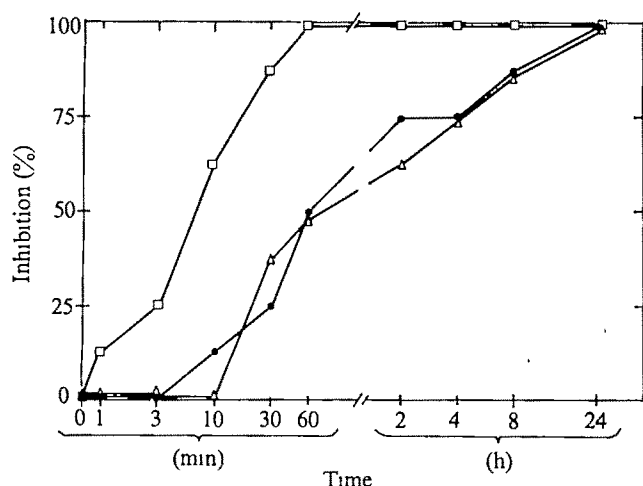


Fig. 1 Inhibition of CPE in T-21, D-21 and M-21 fibroblast cultures exposed to human leukocyte interferon (100 IU ml^{-1}) for varying times (as indicated in the abscissa) and challenged at 24 h with VSV. Human fibroblast cultures, which were either trisomic for chromosome 21 (T-21) (designated SCH), disomic for chromosome 21 (D-21; designated VGS) or monosomic for chromosome 21 (M-21; designated GM-230), were grown to confluency in Eagle's MEM+10% FCS in micro Linbro plates. When confluent, the cells were exposed to 100 IU ml^{-1} human leukocyte interferon (in Eagle's MEM+3% FCS) for either 1, 3, 10, 30 or 60 min, or 2, 4, 8 or 24 h. After incubation with interferon the cells were drained, washed and further incubated with Eagle's MEM+3% FCS. At 24 h all cell cultures were challenged with 100 CCID₅₀ (cell culture infecting dose 50) of VSV and viral CPE readings were made as soon as CPE reached 100% in the virus controls: this was, for a large number of experiments, at 1 d in GM-230 cells, at 2 d in VGS cells and at 2–3 d in SCH cells. □, T-21 (SCH); ●, D-21 (VGS); △, M-21 (GM-230).

If chromosome 21 indeed codes for the interferon receptor, one may expect T-21 cells to be more sensitive to all activities of interferon, both antiviral and non-antiviral. Our data indicate, however, that, unlike the antiviral activity, the non-antiviral effects of interferon "priming" and "toxicity enhancement" are not better expressed in T-21 than in normal diploid cells. These findings are difficult to reconcile with an interferon receptor coded for by chromosome 21, unless one assumes the existence of two (or more) interferon receptor sites, one of which is responsible for the antiviral state and is controlled by chromosome 21, and a second one which is not determined by chromosome 21 and mediates (some of) the non-antiviral effects of interferon.

If the assumption that chromosome 21 codes for an interferon receptor is correct, one might expect cellular binding of human interferon to increase in the order M-21, D-21 and T-21, the more since binding of interferon to the cells seems to be related to the antiviral and anticellular action of interferon in these cells^{11–13}. Therefore, cell binding of interferon was examined with M-21, D-21 and T-21 cells, and, to minimise nonspecific binding, the cells were exposed to interferon for a limited time period (30 min). In concomitant experiments it was ascertained that such short incubation enabled part of the antiviral potency of interferon to be expressed.

The sources of human leukocyte and fibroblast interferon were the same as those referred to previously¹. All human fibroblast cultures were derived from skin biopsies. The monosomic 21 lines (designated GM-137 and GM-230) were obtained from the Mammalian Genetic Mutant Cell Repository (Camden, New Jersey). The latter cell lines were also employed in Tan's studies². All cell cultures were matched as nearly as possible with regard to the number of cell generations in culture.

To measure resistance to virus infections, cells are generally incubated with interferon for a 18–24-h period before virus challenge. In such conditions T-21 fibroblasts are more

sensitive to the antiviral action of interferon than normal diploid fibroblasts, which are in turn more sensitive than M-21 fibroblasts^{2,4}. When the time of exposure of interferon to the cells was shortened, interferon activity gradually diminished (Fig. 1). Irrespective of the time the cells were left in contact with interferon before virus challenge, T-21 cells proved invariably more sensitive to the antiviral activity of interferon than D-21 or M-21 cells—for example, a 30-min exposure of T-21 cells to 100 IU ml^{-1} of interferon afforded nearly complete protection against the cytopathic effect of vesicular stomatitis virus (VSV). To attain a similar protection in D-21 and M-21 cells, an 8-h interferon treatment was required (Fig. 1). Contrary to what may have been expected from a gene dosage effect², M-21 and D-21 cells were equally sensitive to the antiviral action of interferon (Fig. 1). It should be pointed out that viral cytopathic effect (CPE) developed faster in M-21 than in D-21 cells (whether the differences in rate of CPE development reflect differences in the kinetics of virus multiplication is now being studied). Therefore, CPE readings were made at an earlier time for M-21 than for D-21 and T-21 cells. This may explain the differences between our results and those of Tan², who estimated viral RNA synthesis for all cell types at the same time (8 h after infection).

In T-21 cells, the same interferon titre was attained whether the cells were inoculated with VSV 24 h or immediately after a 30-min interferon treatment. Such a short interferon pulse, whether carried out at 37 (Table 1) or 4 °C (data not shown) resulted in the expression of 3–10% of the total interferon activity in T-21 cells and 0.3–1% of the total interferon activity in M-21 and D-21 cells (Table 1). Again, in conditions in which virus challenge was applied immediately after a 30-min interferon treatment, no significant differences were observed in the behaviour of D-21 and M-21 cells. Thus, no gene dosage effect² could be observed in our assay conditions.

Human fibroblast cultures derived from old donors behave very much like T-21 cells in their sensitivity to the antiviral action of interferon¹⁴. The differential sensitivity

Table 1 Interferon titre expressed in various T-21, D-21 and M-21 fibroblast cultures exposed to human leukocyte interferon or human fibroblast interferon (10^4 IU ml^{-1}) for 30 min at 37 °C, and then challenged immediately with VSV

Cell type	Designation	Interferon titre ($\log_{10} \text{ IU ml}^{-1}$)	
		Leukocyte interferon	Fibroblast interferon
T-21	SCH	2.8 (2.5–3.0)	2.5
	BR	3.0	2.5
Old D-21	SW	2.5	2.3
	G	2.5	2.3
D-21	VGS	1.8 (1.5–2.0)	1.6 (1.5–1.8)
	VG ₂ S	2.0	1.5
M-21	GM-230	1.7 (1.5–2.0)	1.5
	GM-137	2.0	1.5

Human fibroblast cultures, which were either trisomic for chromosome 21 (T-21; designated SCH and BR), disomic for chromosome 21 (D-21) and derived from old donors (designated SW and G, both 80 yr old), disomic for chromosome 21 (D-21) and derived from young donors (designated VGS and VG₂S), or monosomic (M-21) for chromosome 21 (designated GM-230 and GM-137), and which had been grown to confluency in micro Linbro plates, were exposed to serial dilutions (in Eagle's MEM+3% FCS) of human leukocyte or fibroblast interferon stock preparations, both containing 10^4 IU ml^{-1} . The interferon incubation period was limited to 30 min at 37 °C. Immediately afterwards, the cells were washed and challenged with 100 cell culture infecting dose 50 (CCID₅₀) of VSV. The interferon titre was determined as the reciprocal of sample dilution inhibiting viral CPE by 50%. CPE readings were taken as soon as viral CPE in the control cell cultures reached 100%. The data show means for 3–10 experiments. The range of the individual values is indicated in parentheses.

Table 2 Binding of interferon to T-21, D-21 and M-21 fibroblast cultures exposed to human leukocyte interferon or human fibroblast interferon for 30 min at 37 °C

Interferon applied to the cells (log ₁₀ IU ml ⁻¹ per Petri dish)	Interferon recovered from culture medium (log ₁₀ IU ml ⁻¹ per Petri dish)			Interferon recovered from cells (log ₁₀ IU ml ⁻¹ per 5 Petri dishes)		
	T-21	D-21	M-21	T-21	D-21	M-21
Human leukocyte interferon						
3.0	2.8	2.8	2.8	1.2	1.2	1.0
4.0	3.6	3.6	3.3	1.4	1.4	1.3
5.0	5.2	5.2	5.2	2.7	2.5	2.5
Human fibroblast interferon						
3.0	2.5	2.2	2.5	1.0	1.0	0.7
4.0	3.4	3.3	3.3	1.2	1.1	1.0
5.0	4.5	4.5	4.5	1.5	1.7	1.3

Human fibroblast cultures, which were either trisomic for chromosome 21 (T-21; designated SCH), disomic for chromosome 21 (D-21; designated VGS) or monosomic for chromosome 21 (M-21; designated GM-230) and which had been grown to confluency in 60-mm Falcon Petri dishes, were exposed to human leukocyte or fibroblast interferon (10³, 10⁴ or 10⁵ IU ml⁻¹ in Eagle's MEM+3% FCS). The interferon incubation period was limited to 30 min at 37 °C. Immediately afterwards, the culture medium was collected for assay of residual interferon activity and the cell monolayer was washed three times with Dulbecco's PBS at 4 °C. The cells were then scraped off with a rubber policeman into 0.5 ml PBS per Petri dish, pelleted and resuspended with 1 ml PBS per 5 Petri dishes. Cell homogenates were prepared by ultrasonication for 20 s in a 100 W ultrasonic disintegrator MSE (operating at maximum output and at a nominal frequency of 20 KHz). These cell homogenates were assayed for interferon content. All interferon titrations were carried out using a VSV CPE inhibition assay in D-21 (VGS) cells. Approximate number of cells per Petri dish: 10⁶ (for T-21, D-21 and M-21 cells). Average protein content per Petri dish (determined by the Lowry method): 852 µg (range: 550–1,080 µg) for T-21, 985 µg (range: 640–1,240 µg) for D-21; and 625 µg (range: 520–720 µg) for M-21 cells.

of 'old' and young' diploid fibroblasts to the antiviral effect of interferon was also observed in the present experiments in which the cells were pulse-treated with interferon before virus challenge (Table 1).

The rapid action of human interferon in T-21 and old D-21 cells (Table 1) is reminiscent of the rapidly developing resistance to virus infection induced by interferon in what has been considered to be physiological conditions¹⁴.

Is the enhanced antiviral action of interferon in T-21 cells, as noted after a brief exposure of the cells to interferon, mediated by or, at least related to an increased binding of interferon to the cells? To assess this question, cell binding of interferon was examined in conditions which allowed a clear differentiation between the antiviral activities of interferon in T-21 and D-21 cells. Therefore, uptake of interferon by the cells was measured after a 30-min contact period at 37 °C. The amount of cell-bound interferon seems to reach a plateau after a 30-min incubation period¹⁵. Cell-associated interferon as well as residual supernatant interferon were quantified for T-21, D-21 and M-21 cells which had been exposed to various doses (10³, 10⁴ or 10⁵ U ml⁻¹) of either human leukocyte or fibroblast interferon. As shown in Table 2, very small amounts of interferon (0.01–0.1%) were taken up by the cells. The amounts of interferon taken up by T-21 cells did not differ markedly from the amount of interferon which became associated with either M-21 or D-21 cells (Table 2).

Thus, the increased sensitivity of T-12 cells to the antiviral action of a 30-min interferon pulse is not reflected by an increased binding of interferon to these cells. Such increased binding would have indicated an increased number of available interferon receptors and/or an increase in the affinity of such receptors for interferon. Provided that our data do not support the existence of an interferon receptor site coded for by chromosome 21, how could they be reconciled with the implication of a chromosome 21-specified cell surface component in the antiviral action of interferon? Hypothetically, the cellular component coded for by chromosome 21 is not the interferon receptor *per se*, but part of a molecular complex that is involved in the processing of the antiviral message from the cell surface to the interior of the cell. The regulator function of chromosome 21 would be required for the antiviral action of interferon, but not for its other effects such as "priming" and "toxicity enhancement"¹⁶. Since D-21 cells derived from old donors (ref. 14 and Table 1) and some other D-21 cells (for example, cells derived from cystinosis patients⁴) behave like T-21 cells in their sensitivity to the antiviral action of

interferon, whereas triploid cells (which are trisomic for all chromosomes) act like D-21 cells⁴, the question may even be raised as to whether chromosome 21 directly codes for any protein involved in the interferon response of the cells.

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Episome-like behaviour of donor DNA in transformed strains of *Neurospora crassa*

TRANSFORMATION is a well established process for the transfer of genetic information in bacteria^{1,2}. Although such a system in higher organisms remains to be fully elucidated, certain features of gene transfer in eukaryotes have similarities with bacterial transformation³. The occurrence of inositol-independent (*int*⁺) transformants has been reported in *Neurospora* when an inositol requiring (*int*⁻) mutant strain (89601) is treated with a wild-type (RL3-8A) DNA preparation^{4,5}. Although these transformants are stable for inositol independence (*int*⁺) during the somatic cell cycle they differ in the ability to transmit this character to their sexual progeny. Essentially there are two groups of transformants, one capable of Mendelian transmission

and another showing only a rare non-Mendelian transmission of the transformed character (*int*⁺) (Table 1). Such differences in modes of genetic transmission can be explained if the donor DNA exists as an episome in the transformed strains. Thus the physical status of the episomes determines the Mendelian and non-Mendelian transmission of the genetic character controlled by them. When integrated they are transmitted in a Mendelian way like any other chromosomal gene; whereas in the non-integrated (autonomous) form their nucleolytic degradation (or slow replication followed by segregation) during meiosis can lead to non-Mendelian transmission. This hypothesis is supported by demonstration of a continued aberrant transmission of the inositol independence to the sexual progeny in the subsequent generations³. Here we describe experiments which establish further the validity of the above hypothesis.

We used DNA-intercalating drugs to eliminate the non-integrated episomes from the transformed strains of *Neurospora*. Both ethidium bromide and acridine have been used

Table 1 Summary of the *Neurospora* strains used

Strains	Description
Wild type (RL3-8A)	No growth requirement for inositol (<i>int</i> ⁺)
Mutants (89601 a/A)	Require inositol for growth (<i>int</i> ⁻)
Transformants*	
Group I	
26-2	These strains show Mendelian transmission of the transformed character (<i>int</i> ⁺) to their meiotic progeny
165	
169	
Group II	
26-6	These strains are rarely able to transmit their <i>int</i> ⁺ character to their sexual progeny and thus show non-Mendelian transmission
166	
Others	
26-6-1	Require inositol for growth (<i>int</i> ⁻) and were obtained after treatment of the transformed strain 26-6 with ethidium bromide (10 µg ml ⁻¹)
26-6-2	
26-6-3	

*These strains were obtained as inositol-independent (*int*⁺) colonies after treatment of the mutant strain (89601) with the wild-type (RL3-8A) DNA preparation^{4,6}. These strains also carry another genetic marker (*os*⁻) and cannot grow on 1 M NaCl. Their origin has been described elsewhere⁵.

successfully to induce loss of autonomous episomes from bacteria⁷⁻⁹ and their mode of action is well known¹⁰⁻¹¹. The drug-induced loss of episomes in *Neurospora* would lead to the production of cells requiring inositol for growth; such *int*⁻ colonies can be detected easily in *Neurospora* by conidial analysis. When treated with these drugs, however, only transformants showing non-Mendelian transmission of the *int*⁺ character would be expected to yield *int*⁻ colonies, whereas the wild-type strain and the transformants showing Mendelian transmission would remain unaffected. Following this rationale, the different *int*⁺ strains of *Neurospora* were treated with the drugs. The results of the experiments presented here support the hypothesis of episome-like behaviour by the donor DNA in the *int*⁺ transformants.

The strains of *Neurospora crassa* used are described in Table 1. Cultures of *int*⁺ strains were treated with different concentrations (0–100 µg ml⁻¹) of ethidium bromide or acridine. The latter was a mixture of 2,8-diamino-10-methyl acridinium chloride and 2,8-diamino acridine (Sigma). The drug was added to a culture at its inception and each culture was then incubated for 4–5 d in the dark at 25 °C. The mycelial mat thus obtained was washed thoroughly and transferred to complete agar medium and allowed to conidiate by incubation for a further 4–5 d. Conidia from each culture were collected separately and plated on complete agar medium containing L-sorbose¹². The growing colonies were then isolated and tested for growth requirement by spreading on minimal medium and on medium supplemented with inositol (80 µg ml⁻¹). The colonies able to grow on minimal medium were scored as *int*⁺ (their

growth was not affected by the presence of inositol in the medium). Those which required inositol for growth and could not grow on the minimal medium were scored as *int*⁻. The frequency of production of *int*⁻ colonies was considered to be a direct measure of the drug-induced loss of the *int*⁺ determinants. The data presented in Tables 2 and 3 show that the drugs produced *int*⁻ colonies in significant numbers only from the transformants which showed a non-Mendelian transmission of the transformed character (that is group II). Ethidium bromide was effective in this respect on both strains 26-6 and 166 of this group (Tables 2 and 3). The data in Table 2 also suggest that the loss of inositol independence depended on the concentration of ethidium bromide. The frequency of production of *int*⁻ colonies increased linearly with increasing drug concentration (Table 2): 50 µg ml⁻¹ was found most effective, and approximately 8% of treated colonies were *int*⁻.

The effect of acridine was similar to that of ethidium bromide. The data in Table 3 show the production of *int*⁻ colonies in a significant number by the group II transformants (strains 26-6 and 166) treated with acridine. The effect of acridine also depended on the concentration of drug used (data not shown). The drug-induced loss of the *int*⁺ character among the group II transformants suggests that in these transformants the donor DNA exists as an autonomous episome (which can be eliminated by treatment with DNA-intercalating drugs).

For comparison, the group I transformants (that is, strains 26-2, 165 and 169) were treated with ethidium bromide and acridine. The data in Table 3 show that their inositol independence was unaffected by drug treatment; no colony which required inositol for growth was produced by such treatment. These data suggest that in the group I transformants the donor DNA (carrying the *int*⁺ genetic information) has been integrated into the recipient chromosome. As another control, the wild-type strain (RL3-8A) was treated with both of the drugs and, among a large number of the colonies examined, all were found to grow on minimal medium (Table 3). Thus the wild-type strain could retain its inositol independence (*int*⁺) after drug treatment as expected since the *int*⁺ genetic information is chromosomal in this strain. The fact that the inositol independence of the wild-type strain was not affected by the drug rules out the possibility of a mutagenic effect of the drug on other strains. Crosses were performed to establish the allelism of the newly isolated *int*⁻ strain (26-6-1, 26-6-2, and 26-6-3, Table 1) with the parental *int*⁻ strains (89601). The new *int*⁻ strain in crosses with 89601 (*int*⁻) produced only *int*⁻ progeny; not one wild-type progeny was detected even when more than a million spores were tested from each of these crosses. Also the new *int*⁻ strains in crosses with the wild-type strain were found to produce asci with four *int*⁻ and four *int*⁺ spores per ascus. These genetic data

Table 2 Ethidium bromide-induced loss of the inositol independence (*int*⁺) in a transformed strain (26-6) of *Neurospora crassa*

Drug concentration (µg ml ⁻¹)	No. and phenotype of colonies obtained after the drug treatment			Frequency of the colonies which lost <i>int</i> ⁺ character (%)
	<i>int</i> ⁺	<i>int</i> ⁻	Total	
0	886	0	886	0
1	500	0	500	0
5	494	6	500	1
10	581	17	598	2.9
20	473	27	500	5.4
50	459	41	500	8
100	463	37	500	7.5

The transformed strain 26-6 was treated with the different concentrations of ethidium bromide and the colonies obtained by the conidial analysis were examined for their growth requirement with respect to inositol (see text).

Table 3 Effect of drug treatment on the loss of inositol independence (*inl*⁺) among different strains of *Neurospora crassa*

Strains	No. and phenotype of colonies examined after treatment with Ethidium bromide* (10 µg ml ⁻¹)			Acridine* (15 µg ml ⁻¹)		
	<i>inl</i> ⁺	<i>inl</i> ⁻	Total	<i>inl</i> ⁺	<i>inl</i> ⁻	Total
Wild-type strain (<i>inl</i> ⁺) (RL3-8A)	2,560	0	2,560	2,700	0	2,700
Transformed strains (<i>inl</i> ⁺)						
(I) showing Mendelian transmission of the <i>inl</i> ⁺ character						
26-2	943	0	943	500	0	500
165	842	0	842	900	0	900
169	921	0	921	800	0	800
(II) Showing non-Mendelian transmission of the <i>inl</i> ⁺ character						
26-6	662	18	680	1,649	41	1,680
166	689	11	700	587	13	600

*The different inositol-independent (*inl*⁺) strains of *Neurospora* were treated with the drugs separately and then examined by conidial analysis as described in the text.

clearly establish that the *inl*⁻ phenotype of the drug-treated culture was determined by a Mendelian gene and that latter was allelic to the parental *inl*⁻ strain (89601).

An episome can exist inside the recipient cell in two distinct forms, the autonomous state and the integrated state¹³, and the data presented here are compatible with the hypothesis that donor DNA exists as an episome in the transformed strains of *Neurospora*. These data suggest the difference in the physical status of the donor DNA (carrying the *inl*⁺ genetic information) in the two groups of the *Neurospora* transformants. In the group I transformants the episome is chromosomal whereas in group II it is autonomous. Although acridines are known mutagens¹⁴, the effect of ethidium bromide and acridine reported here cannot be explained on the basis of a mutation at the *inl*⁺ locus in the transformants because these drugs did not produce *inl*⁻ colonies in the wild-type strain. Because the transformed strains were stable during their somatic cell cycle it seems that the donor DNA may exist as covalently closed circles to avoid nucleolytic digestion. These, however, could still be liable to digestion by restriction endonucleases during meiosis; the existence of such endonucleases in higher organisms has been invoked to provide the molecular basis of non-Mendelian inheritance in higher organisms^{15,16}. The frequency of elimination of episomes carrying *inl*⁺ genetic information in *Neurospora* was rather low (8%) compared with the high frequency of elimination (up to 100%) of F factors reported earlier¹⁷. These values in *Neurospora*, however, are comparable with that of the elimination of the drug resistance factors in bacteria¹⁸.

The data presented here imply certain similarities in the behaviour of episomes in prokaryotes and eukaryotes as suggested previously¹⁹. Some of the characteristics of genetic transformation in *Neurospora* described here have some parallels in other eukaryotes²⁰.

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Genetic polymorphism of C4 in man and localisation of a structural C4 locus to the HLA gene complex of chromosome 6

NATIVE C4, the fourth component of human complement (C4¹) has a molecular weight of 204,000 and is composed of three distinct polypeptide chains linked by disulphide bonds and non-covalent forces². C4 migrates as a β 1 globulin when subjected to electrophoresis in Agarose gel. Genetically determined structural polymorphism has been detected in the complement components C3 (refs 3 and 4), C6 (ref. 5) and properdin factor B (ref. 6). As well as their use in forensic science and population genetics, such polymorphisms are valuable tools for work on genetic linkage and chromosome mapping, as shown by the assignment of the factor B locus to chromosome 6, closely linked to HLA-B*¹⁸. We report here similar findings with human C4.

EDTA-plasma, heparin-plasma and serum were used initially. Samples were either studied within 2 h of collection or stored at -75 °C until examined. Serum proteins were separated by high voltage electrophoresis in Agarose gel⁹ for 1-1.5 h. C4 bands were then made visible by immunofixation with specific antibody¹⁰, and staining with Coomassie brilliant blue. Specific anti-C4 was produced by immunising rabbits with C4 purified by ion exchange chromatography, ammonium sulphate precipitation and preparative electrophoresis on polyacrylamide gel. This monospecific anti-C4 showed reactions of complete identity with anti-C4 obtained from Dr C. Alper, Boston, and also with commercial anti-C4 (Behring Institut and Atlantic Antibodies).

Ninety-three unrelated Norwegian adults have been studied. In 91 of them, one of three patterns was found: one slow (S) band only, one fast (F) band only, or the two together (FS) (Fig. 1). In two individuals a variant pattern was observed in which the ordinary F band occurred together with a band (M) localised between the position of

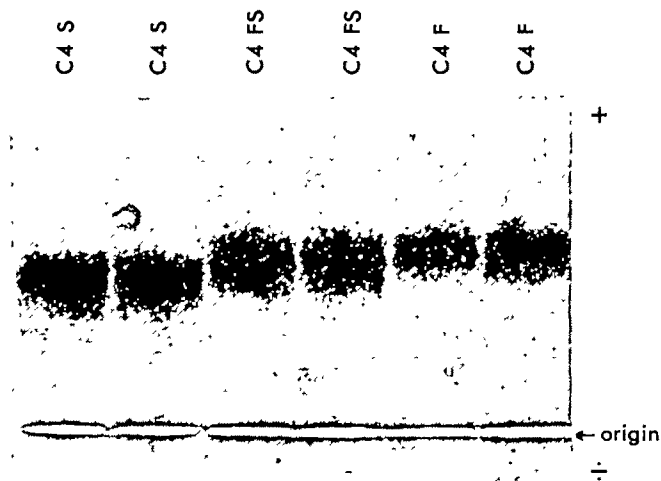


Fig. 1 The common C4 phenotypes revealed by immunofixation electrophoresis.

the S and F bands. These patterns could be reproduced in the same samples examined on different occasions, and in samples from the same individuals bled at different times. The patterns were identical in serum, EDTA-plasma and heparin-plasma when these were handled as described above.

The family data consist of 17 matings with 112 children. The families have been typed for approximately 20 other genetic marker systems, thus assuring a regular pattern of inheritance. Heparin-plasma samples stored at -75°C were typed and the results confirmed the postulate that the bands S and F observed in the material of unrelated adults represent the expression of two alleles $C4^S$ and $C4^F$ at one locus—that is a structural locus for C4 (Table 1).

Among 93 unrelated Norwegians the following gene frequencies were observed: $\sim 0.52 C4^S$, $\sim 0.47 C4^F$ and $\sim 0.01 C4^M$. Phenotype frequencies were in agreement with the expected Hardy-Weinberg distribution (23 C4S, 2 C4FM, 51 C4FS and 17 C4F). Preliminary findings with an extended population sample suggest the existence of other variant alleles.

Table 1 C4 types in 17 matings with 112 children

Mating type	No. of matings	No. of children	C4 types of children				
			S	FS	F	FM	MS
S \times FS	2	22	8	14			
S \times F	1	4		4			
FS \times FS	2	6		4	2		
FS \times F	9	60		25	35		
F \times F	2	8			8		
FS \times FM	1	12		3	2	3	4
	17	112	8	50	47	3	4

The heterogeneity of human C4 has been reported before^{11,12}, and has been postulated to have, at least in part, a genetic background. Gene frequencies, however, have not been reported, and no extensive family studies have been done. It is therefore difficult to assess whether this heterogeneity is related to the polymorphism described here.

The detection of a structural polymorphism gave us the opportunity to try and link the C4 locus to other loci of the human genome. In 85 HLA-B/C4 informative meioses there were no recombinations. This definitely proves that the two loci are closely linked, the recombination fraction being 0.0–4.3 (95% confidence limits). Two informative families with known crossovers between the A and B loci of the HLA region, moreover, indicate that the C4 locus is situated close to the B locus, as shown for the properdin factor B locus⁴.

A patient has been reported with no C4 in the serum, and

a study of the family suggested that the locus determining the level of C4 was closely linked to the HLA region¹³. Our findings suggest that the familial defect in C4 levels is due to a "silent" allele at the structural C4 locus.

The structural locus of properdin factor B, and loci determining the blood levels of C2 (ref. 14) and C8 (ref. 15) have been assigned previously to the HLA gene complex of chromosome 6. It is therefore not surprising that C4 is also encoded by a locus in this complex. It has been shown that the murine C4 locus is situated in the H-2 gene complex^{16,17}. The C3 locus in man is, however, not linked to HLA¹⁸.

In this context it is tempting to speculate that loci for different components of complement have appeared, during evolution, from one locus as results of local chromosomal events, for instance gene duplications and point mutations. Indeed it has been postulated that complement components have evolved from a single ancestral protein².

An interesting pattern emerges with respect to the role of this short segment of chromosome 6 in the immunobiology of vertebrates. It seems significant that many important factors in immune recognition, response and reaction are encoded by closely linked genes. The closeness at the chromosomal level may reflect functional integration of products of immune response genes, cell membrane allo-antigens and the complement system.

Note added in proof: Recently extended population material indicates that the frequency of the S gene may be appreciably lower than in the small sample presented here (~ 0.40).

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Persistence of anti-immunoglobulin on the lymphocyte surface

It is well established that addition of anti-immunoglobulin (anti-Ig) to lymphocytes expressing Ig receptors produces "capping" followed by endocytosis or "sloughing" of the membrane Ig, the expression of which is greatly diminished until resynthesis occurs¹. Another property of anti-Ig in some species, notably the rabbit, is blastogenic activation of lymphocytes². It is known that for the activation of lymphocytes by plant mitogens, the mitogenic substance must persist on the cell membrane for several hours before irreversible activation of the cell occurs³. Similarly, the activation of rabbit lymphocytes by antiserum to allotypic Ig determinants is largely inhibited if the Ig expressing the target allotype is added within a few hours⁴. Thus there is an apparent conflict between these two phenomena, one of which centres around the rapid removal of membrane Ig, the other requiring the persistence of anti-Ig for longer periods. Accordingly, experiments were designed to test the degree of retention of anti-Ig and the re-expression of membrane Ig on the surface of lymphocytes treated with anti-Ig sera and retained in tissue culture for several days.

To study the fate of anti-allotype antibody on rabbit lymphocyte membranes *in vitro*, peripheral blood lymphocytes, homozygous for *b*-locus allotype determinants, were used. These lymphocytes were obtained from defibrinated blood by a gelatin sedimentation technique⁵. In all cases the cell suspensions were incubated at 37 °C for 1 h to reduce the level of cytophilically bound immunoglobulin. The lymphocytes were suspended in 1 ml of Eagle's MEM (Glasgow modification) supplemented with 10% heat inactivated FCS (MEM-F) to a cell concentration of 2×10^6 ml⁻¹. An appropriate volume (0.03 or 0.06 ml) of anti-allotype serum was added and the cells cultured for 24 h at 37 °C in 10% CO₂ in air. The cells were spun down, residual antiserum removed and the cells washed once in warm MEM-F, before reculturing in antibody-free MEM-F for 0, 1, 24 and 48 h. At each time period the cells were washed three times (finally in PBS) and fixed in 2% paraformaldehyde solution⁶. At the end of the experiment the fixed cells were tested for the presence of allotypic determinants characteristic of the membrane Ig and of the attached antibody by a mixed antiglobulin test (see Table 1). The allotypic determinants are known to survive the fixation procedure⁷ whereas antibody activity is destroyed. A rosette assay was chosen because it has the unique advantage of detecting surface Ig and excluding intracellular Ig.

The fate of the anti-allotype antibody and of the membrane Ig receptors is shown in Table 1. Anti-allotype antibody was readily detectable on lymphocyte membranes immediately after 24 h of incubation with antiserum and persisted on approximately the same number of cells for a further 2 d. A significant number of the cells bearing antibody appeared to be blast cells (see parentheses, Table 1). Little of this antibody seems to be taken up cytophilically on to Fc receptors, since similar results were obtained with an F(ab')₂ anti-allotype preparation. The rapid disappearance from the cell membrane of any Ig taken up from a normal b6 serum provides further evidence that the persistence of antibody is unlikely to be due to cytophilic binding (Table 2). However, as demonstrated using normal b6 serum, some cytophilic uptake of Ig does take place during the initial 24 h incubation, which appears to be greatly reduced 1 h after washing and effectively lost by 24 h in culture. This cytophilic uptake appears to be entirely by the Fc receptor since it did not occur with the F(ab')₂ preparation.

Immediately after the initial incubation the washed lymphocytes exhibited high levels of bound antibody, but

low levels of membrane Ig. After 24 h incubation most of the cells appeared to recover their membrane Ig which was maintained over a further 24 h in culture. The proportion of blast cells expressing membrane Ig was approximately the same as that on which the anti-allotype antibody was present.

The observation that complexes appear in the supernatants of cultured mouse lymphocytes after only 2 h of incubation with anti-Ig⁸ prompted an experiment to investigate the possible nonspecific uptake by lymphocytes of "shed" membrane Ig-anti-Ig complexes. For this experiment b9b9 lymphocytes were cultured in a supernate (presumed to contain b4 m-Ig-anti-b4 complexes) obtained after a 3-h incubation of b4 cells with antibody. The cells were fixed after 1, 18 and 42 h and rosetting performed. Less than 1% (3 in 500 cells) of the cells formed rosettes (and these seemed to be macrophages), indicating that cytophilic up-

Table 1 Anti-Ig antibody and membrane Ig on cultured lymphocytes after prior exposure to anti-Ig serum

Experiment no.	Anti-allotype serum present during first 24-h culture (final concentration)*	Reculture time (h)	Lymphocytes (%) bearing membrane-Ig or antibody-Ig	
			Membrane-Ig	Ab-Ig
1	None (control)	0	50	—
		24	70	—
		48	70	—
	Anti-b4 (b5b6) (1 in 30)	0	7	46
		1	8	30
		24	53 (3)	32 (2)
	Anti-b4 (b5b6) (1 in 15)	0	17	53
		1	17	38
		24	64 (21)	45 (17)
		48	65 (39)	69 (31)
2	None (control)	0	37	—
		24	51	—
		48	60	—
	Anti-b4 (b5b6) F(ab') ₂ preparation (100 µg ml ⁻¹)	0	2	27
		1	6	30
		24	35 (9)	37 (12)
		48	34 (12)	44 (18)
3	None (control)	0	44	—
		24	57	—
		48	61	—
	†Anti-b5 (b6b6) (1 in 15)	0	1	35
		1	0	24
		24	28	23
		48	39	29
4	None (control)	0	46	—
		24	47	—
	Anti-b6 (b4b4) (1 in 30)	0	39 ²⁶ (c)	46
			13 (r)	
		24	34 ¹⁰ (c)	28 (2)
			24 (r)	
		48	51 ⁶ (c)	49 (14)
			45 (r)	

Briefly the method for the mixed anti-globulin rosette assay⁸ was as follows. The fixed lymphocytes were mixed with a 1/50 dilution of anti-allotype serum (either anti-b4 or anti-b6) for 30 min at room temperature and washed. SRBC (5% suspension) sensitised with a 1/60 dilution of either b4 or b6 rabbit anti-SRBC Fab⁹ preparation for 30 mins at 37 °C and washed. Sensitised SRBC (0.06 ml of a 1% suspension) and the sensitised lymphocytes (0.06 ml of 2×10^4 ml⁻¹) were mixed and centrifuged at 200g for 2 min. The cell pellet was gently resuspended and Toluidine blue added (0.03 ml of a 0.2% solution). Lymphocytes binding four or more RBC were counted as positive and expressed in the table as the mean of duplicate tests. Appropriate specificity controls were included to test for the non-specific uptake of the sensitising antibody by paraformaldehyde fixed cells. No detectable uptake of an irrelevant antiserum (that is anti-b6 on b4b4 cells) was ever observed.

*All anti-allotype sera were used at optimum concentrations for lymphocyte stimulation. Lymphocytes of allotype b4b4 were used in experiment 1, 2 and 3, and of b6b6 in experiment 4.

†Anti-b5 showed cross reactivity with b4 Ig determinants.

Figures in parentheses denote the percentage of blast cells rosetting. (c) = caps; (r) = rings.

Table 2 Nonspecific uptake of normal serum Ig and of "shed" membrane Ig-anti-Ig complexes by lymphocytes

Experiment no.	Initial culture composition and duration	Reculture time (h)	Lymphocytes (%) positive for Ig of extrinsic type
1	b4b4 lymphocytes cultured 24 h with b6b6 normal serum (1 in 15)	0	21
		1	6
		24	1
		48	3
2	b9b9 lymphocytes cultured with supernate from b4 m-Ig-anti-b4 culture	1	<1
		18	<1
		42	<1

Rosetting was performed as described in the legend to Table 1. For experiment 2, approximately 12×10^6 lymphocytes (Ficoll-trisoli purified) were cultured at $6 \times 10^6 \text{ ml}^{-1}$ in MEM-F with 0.06 ml anti-b4 (b5b6) serum for 30 min at 4 °C. The cells were washed once in the cold, then cultured at 37 °C for 3 h in MEM-F. The supernates (0.6 ml) were then transferred to 2×10^6 b9b9 lymphocytes. After 1, 18 and 42 h at 37 °C, the cells were washed and fixed in paraformaldehyde and rosetting performed.

take of complexed antibody is not a significant source of cells expressing allotypic antibody determinants.

The experiments described demonstrate that some anti-allotype antibody persists for at least 48 h in culture after it has become fixed to the cell surface. This is in apparent conflict with the efficient "capping" of fluorescein-labelled antibody which has been shown to occur in this system⁸. There is also evidence in the experiments described here for the "capping" of antibody in the form of "capped rosettes". The explanation of these findings is that a part, perhaps a large part, of the antibody attached to membrane Ig is removed relatively rapidly by "capping" but the remainder persists for very long periods. Alternatively, antibody may persist as a result of binding to resynthesised membrane Ig. The fact that antibody persists on the surface well beyond 24 h is compatible with the induction of activation by anti-allotype serum. These results are consistent with findings for the fate of concanavalin A (con A) and phytohaemagglutinin (PHA) in lymphocyte cultures where about 70% of the surface-bound lectin has been shown to be rapidly removed, whereas the remainder is retained for longer periods¹⁰.

Lymphocytes do not seem to be unique in the mode of retention of surface-fixed substances. Other workers have demonstrated the persistence of KLH on membranes of macrophages¹¹, antigen-antibody complexes on dendritic cells¹² and DNP-D-GL copolymers on the surface of lymphocytes¹³ for long periods. ALG has also been shown to persist in micro patches on mouse lymphocytes for several hours¹⁴, a fact which is particularly interesting in view of the known lymphocyte-stimulating activity of these reagents¹⁵.

In conclusion, it has been demonstrated that antibody to membrane Ig persists on lymphocyte membranes for up to 3 d in culture. During this period membrane Ig is being resynthesised and blast transformation is taking place.

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Immunodepression, high IgM levels and evasion of the immune response in murine trypanosomiasis

THERE is currently much interest in the devices evolved by parasites which enable them to evade the immune responses mounted by their hosts¹. One such device is the phenomenon of antigenic variation associated with the salivarian trypanosome infections of man and animals in Africa^{2,3}. Typically these infections show successive waves of blood parasitaemia. Each succeeding trypanosome population is characterised by a novel variant-specific glycoprotein antigen present in the parasite surface coat and not reactive with antibodies induced against preceding variants^{4,5}. Antigenic variation has a clear role in the evasion of host immunity and leads to chronic and usually fatal infections. More recently we have become aware of a possible additional component of the evasion mechanism, generalised immunodepression.

Depressed responses to heterologous antigens have been shown in experimental lymphomagenic virus infections^{6,7}, in malaria^{8,9}, and in trypanosome infections^{10,11}. Explanations of the immunodepression in trypanosomiasis are complicated by the concomitant high levels of IgM immunoglobulins, much of

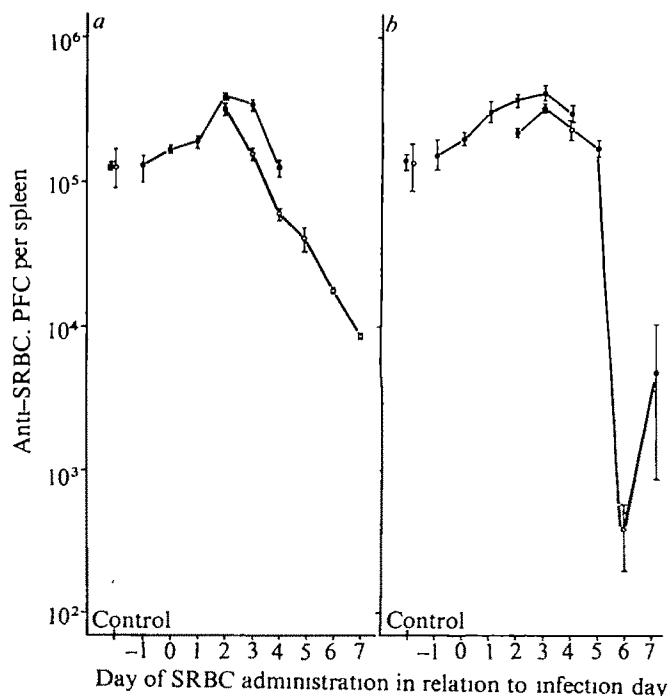


Fig. 1 Altered responsiveness of *T. b. brucei*-infected mice to SRBC. Numbers of PFC in female BALB/c mice given 1×10^6 SRBC intraperitoneally various days before or after infection with *T. b. brucei* S42, 1,000 organisms given intraperitoneally. Each point is the arithmetic mean (± 1 s.e.) of the PFCs per spleen of five mice, and the points represent two overlapping series, series 1 (●), series 2 (○). PFC assayed by the slide method¹². a, Direct (IgM) PFC; b, indirect PFC developed using a rabbit anti-mouse specific IgG_{2a} antiserum titrated and used at a concentration such that the inhibitory constant (KI) and developing constant (KD) = 1.0 (ref. 25).

which appears not to be parasite specific¹²⁻¹⁵. Following a detailed study of the plaque-forming cell (PFC) response to sheep red blood cells (SRBC), and to other heterologous erythrocytes and antigens, in mice infected with *Trypanosoma brucei brucei*, we are able to suggest a possible link between high IgM levels and immunodepression. We further suggest that less than optimal anti-parasite responses result from the immunodepression which thus assists the parasite to evade host immunity.

Figure 1 summarises the data of a number of experiments in which the time intervals between infection and SRBC immunisation were varied. Experimental details are given in the figure and table legends. When SRBC were injected 1-4 d after infection there was enhanced responsiveness, with both IgM and IgG-PFC being 2-3 times those of the uninfected immunised controls. But when SRBC were given 5 or more days after infection, the PFC responses were diminished; at 7 d both IgM and IgG PFC were equal only to background values. This profound depression persisted for the remainder of the infection. Clearly the effect of the infection on the PFC response depends on the temporal relationship between the day of infection and the antigen presentation; responses early in infection may be enhanced whereas later responses are depressed. These findings are broadly similar to the enhancement or suppression of haemolysin production in mice treated with bacterial lipopolysaccharide (LPS)¹⁶. The response was enhanced if LPS was given at the same time or shortly after SRBC, but was largely inhibited if LPS was given 1 or 2 d before the antigen.

We also observed a progressive increase in "background" IgM PFC to SRBC in infected, non-immunised mice. The time course of this background increase is shown in Fig. 2 in relation to the blood parasitaemia. By days 3-4 of infection a pronounced rise is seen which reaches plateau levels by about day 9. Numerous observations indicate that the plateau level of IgM PFC is usually 20-30 times that of uninfected control mice. We next investigated the background PFC of infected mice to two other heterologous erythrocytes, and to three other antigens coated on to SRBCs (Table 1). The values in Table 1 are mainly expressed as PFC per spleen because our interest centres on the total Ig production of the animal. As, however, splenomegaly develops in murine trypanosomiasis, we also express PFC per 10⁶ spleen cells in selected instances (Table 1b). In all cases, background IgM PFC were significantly increased,

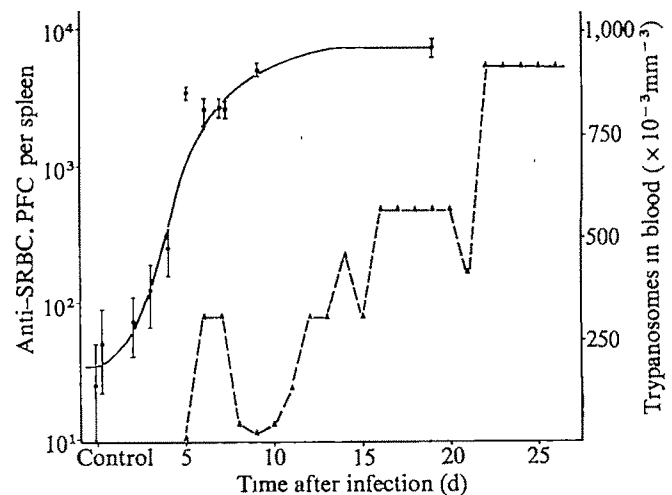


Fig. 2 Rise in SRBC background IgM PFC in *T. b. brucei*-infected mice in relation to blood parasitaemia. Male BALB/c mice were infected with 1,000 *T. b. brucei* S42 intraperitoneally. Each point is the arithmetic mean (± 1 s.e.) of the PFC per spleen of four or five mice. The points represent direct (IgM) PFC in two overlapping series, series 1 (\bullet), series 2 (\circ), all groups within a series being assayed on the same day. Each point on the parasitaemia curve (\blacktriangle — \blacktriangle) represents the mean blood parasitaemia count of 10 mice.

particularly so with the TNP hapten. Because of the number and variety of antigens tested, it seems not unreasonable to assume that the background IgM responses to all antigens recognisable by B cells are increased in trypanosome-infected mice, and that the trypanosome infection therefore induces polyclonal B-cell activation. Indeed, Coutinho and Moller¹⁷ have argued that a marked increase in background IgM PFC to a highly hapten-substituted target cell, such as our TNP-coated SRBCs, is in itself a good measure of polyclonal B-cell activation. They state that such rises, although not specific, reflect increases in the background responses to a variety of cross-reacting determinants. Again a parallel may be drawn between the polyclonal activation of trypanosome infections and that produced by LPS¹⁸. The secreted polyclonal products would then account for the increased Ig levels seen in the infection; by day 14 serum IgM levels had increased 8-16-fold

Table 1 Increase in number of background PFC to a variety of antigens in mice infected with *T. b. brucei*

a PFC per spleen		Day of infection			
Antigens		Controls	8	10	19
SRBC		215 \pm 58	5,380 \pm 779	—	—
SIII	Direct	301 \pm 116	1,128 \pm 230	—	—
CGG	PFC	216 \pm 52	2,652 \pm 700	—	—
TNP		964 \pm 146	31,302 \pm 2,289	—	—
SRBC	Direct	281 \pm 107	—	—	7,000 \pm 1,036
	Indirect	0	—	—	2,391 \pm 598
HRBC	Direct	63 \pm 63	—	—	2,000 \pm 289
	Indirect	0	—	—	756 \pm 222
DRBC	Direct	94 \pm 31	—	—	3,313 \pm 542
	Indirect	0	—	—	1,173 \pm 480
b PFC per spleen and per 10 ⁶ spleen cells					
PFC per spleen					
SRBC	Direct	200 \pm 55	—	11,080 \pm 1,693	—
CGG	PFC	250	—	3,523 \pm 970	—
PFC per 10 ⁶ spleen cells					
SRBC	Direct	4 \pm 0.4	—	29 \pm 5	—
CGG	PFC	4 \pm 0.24	—	9 \pm 2.2	—

Each PFC is the arithmetic mean (± 1 s.e.) of 4-5 mice. Antigens: SRBC, HRBC, DRBC; sheep, horse and donkey erythrocytes: SIII; SRBCs coated with type III pneumococcal capsular polysaccharide using broth culture supernatant¹⁹; CGG; SRBC coated with chicken gamma globulin by incubating equal volumes of 10% SRBC and chicken anti-SRBC antiserum ($\frac{1}{4}$ minimum agglutinating titre) at 37 °C for 30 min. and washing three times: TNP; trinitrophenol determinant coated on to SRBC using TNBS²⁰. The values for SIII, CGG and TNP were calculated according to the formula: PFC.Ag = PFC.Ag SRBC - (PFC.SRBC \times CF). The correction factor (CF) is a measure of the efficiency of lysis of antigen-coated SRBC by anti-SRBC.PFC, and thus reflects the degree of antigen coating. For example in the first series, CF values for SIII, CGG and TNP were 0.46, 0.48 and 0.63. These ratios were established on the day of assay using the same batches of antigen-coated SRBC as in the experimental groups, and were calculated from the geometric means of 10 duplicates using log(x + 1) transformation. The assays were performed on the pooled spleen suspensions from three BALB/c mice given 1×10^6 SRBC intraperitoneally 5 d previously.

and IgG₂ had increased four- to fivefold in these *T. b. brucei*-infected mice (Hudson and Byner, unpublished observations). Table 1 does show increases in background IgG PFC to sheep, horse and donkey RBC, but these increases vary widely from experiment to experiment and never approach the increases seen in background IgM PFC. Polyclonal B-cell activation has previously been suggested to occur in malaria and trypanosomiasis^{19,20}, but hitherto mitogenic activity of these parasites has only been demonstrated *in vitro*^{21,22}.

We believe that the polyclonal activation of B cells in the presence of a continuous trypanosome infection is likely to result in a progressive depletion of antigen-reactive B lymphocytes as these are activated to change into secretor cells, perhaps with little accompanying proliferation. In this way the immunodepression occurring later in the infection may be explained by the depletion of B cells capable of recognising the introduced antigen. Diamantstein and his colleagues²³ have shown suppression of primary responses *in vivo* to SRBC by a number of B-cell mitogens. They believe that when B cells react with a mitogen in the absence of a particular antigen, they temporarily lose their capacity to respond to that antigen. The longer term immunodepression associated with murine trypanosomiasis might then result from the continuous exposure of B cells to trypanosomes and their products.

Finally, if both activation and immunodepression are polyclonal it would be expected that the antibody responses to later-appearing trypanosome antigenic variants would also be impaired. The pattern of infection with S42 *T. b. brucei* (Fig. 2) lends some support to this view. The infection shows waves of parasitaemia which have been shown to be antigenically distinct (K.M.H., C.B., and A. E. R. Taylor, unpublished). The first wave subsides around days 8–9, due to the production of variant specific antibodies. The second major wave, in spite of some minor fluctuations, never subsides to the same degree as the first wave but continues to rise until the death of the mouse. This may perhaps indicate that the production of variant specific antibodies is depressed at this stage of the infection. We are at present attempting to obtain direct evidence on this point and are also investigating a series of more chronic infections. Meanwhile it is a reasonable working hypothesis that high IgM levels, immunodepression of responses to heterologous antigens, and the less than fully effective anti-trypanosome responses are linked, and are brought about by the trypanosomes either directly or indirectly activating the host B cells polyclonally.

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Absence of intestinal mast cell response in congenitally athymic mice during *Trichinella spiralis* infection

DURING the intestinal phase of nematode infections in laboratory rodents a variety of cell types is found in the gut mucosa, including mast cells and globule leukocytes^{1–4}. After stimulation by the nematodes present either in the intestinal lumen or penetrated into the gut mucosa, the number of intestinal mast cells increases^{1–4}. Next, mast cells invade the epithelial lining of the villi, discharge secretory products, including histamine, and change into the intra-epithelial globule leukocytes^{1–4}. Release of mast cell secretory products is mediated by IgE antibody⁵. No conclusive evidence has as yet been presented, that the secretory products have a role in the expulsion of the worms.

In earlier studies⁴ the thymus dependence of both intestinal mast cell production and globule leukocyte formation was studied in *Trichinella spiralis* infected rats with and without anti-thymocyte serum (ATS) treatment. ATS treatment did not influence mast cell production, but had an adverse effect on mast cell degranulation which was attributed to a possible decrease in IgE level in the ATS-treated rats. Since in ATS-treated animals a residual pool of T cells can be expected as well as an intact thymus, we repeated these experiments in congenitally athymic (nude) mice and their heterozygous thymus-bearing (+/nu) littermates. In +/nu mice *T. spiralis* adult worms are expelled from the intestinal tract within 10 d after infection, whereas in nude (nu/nu) mice worms are present until at least 50 d after infection^{7,8}, indicating T-cell dependence of host protection against a *T. spiralis* infection.

Male SPF B10LP nude (nu/nu) mice, 6 weeks of age, were obtained from the Central Laboratory for the Breeding of Laboratory Animals TNO, Zeist, The Netherlands, where the mice were maintained by conventional back crossing with strain B10LP. Comparisons were made with age-matched mice heterozygous for the nude gene (+/nu). At an age of 8 weeks the mice were orally infected with 100 *T. spiralis* larvae each, using a syringe. Animals were autopsied from 3 to 16 d after infection. The presence of intestinal mast cells and globule leukocytes was studied in a 10-cm portion of the proximal jejunum with a swiss-roll technique⁹. Tissues were fixed in a special fixative, adopted after preliminary studies, since intestinal mast cells differ in their staining properties from tissue mast cells elsewhere in the body and the fixative proposed for rat intestinal mast cells¹⁰ proved inadequate. The fixative was composed of a mixture of 0.8% formalin buffered with phosphate-buffered

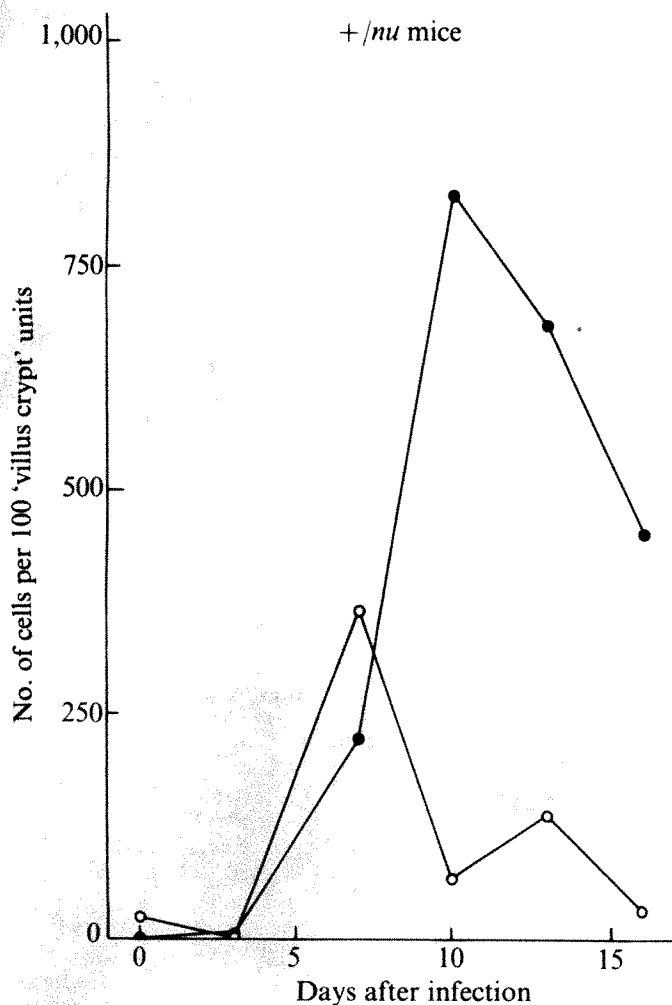


Fig. 1 Mean number of intestinal mast cells and globule leukocytes in a 10-cm portion of the proximal jejunum (expressed per 100 "villus crypt" units) in groups of 4-5 $+/\nu$ mice infected orally with 100 *T. spiralis* larvae each. Statistical analysis (Student's *t* test). Mast cells: day 3 against day 0, $P < 0.001$; day 7 against day 0, $P < 0.01$; other days after infection against day 0, not significant. Globule leukocytes: day 3 against day 0, not significant; day 7 against day 0, $P < 0.001$; day 10 against day 0, $P < 0.001$; day 13 against day 0, $P < 0.01$; day 16 against day 0, $P < 0.001$. \circ , Mast cells; \bullet , globule leukocytes.

saline (0.01 M; pH 7.2) at neutral pH and 4% acetic acid. After overnight fixation at 45 °C tissues were dehydrated and embedded in paraplast according to conventional procedures. Sections (5 μ m thick) were prepared and stained with toluidine blue for 20 min at room temperature. Toluidine blue (0.5%) was dissolved in 20% ethanol. After filtration the solution was used for staining. After staining the sections were washed, dehydrated and mounted with a conventional mounting medium.

Cells present in the intestinal mucosa were counted in the whole swiss-roll and their number expressed per 100 "villus-crypt" units. A "villus-crypt" unit represents a portion of gut mucosa, lying between two gland crypts and the lamina propria of the villus above¹¹. Non-*T. spiralis*-infected animals served as controls (day 0 in Figs 1 and 2).

The results of the mast cell and globule leukocyte counts in the $+/\nu$ mice are shown in Fig. 1, the corresponding counts for the ν/ν mice in Fig. 2.

In $+/\nu$ mice, the number of intestinal mast cells decreased at day 3 after infection and showed a rise at day 7, after which a gradual decline was observed. The number of globule leukocytes increased noticeably from day 7 to day 10 after infection; at days 13 and 16 a decline was seen. In ν/ν mice, mast cells and globule leukocytes were present at day 0, although the number was much lower

than that found in $+/\nu$ mice. During the course of the infection no change in number of either cell type was observed.

The intestinal mast cell response in $+/\nu$ mice corresponds with that described for rats infected with *T. spiralis*⁴ or *Nippostrongylus brasiliensis*⁵. The initial decrease in number of mast cells is attributed to toxic metabolites secreted by the parasite¹². The subsequent increase in number of intestinal mast cells has been tentatively attributed to proliferation of precursor cells of lymphoid origin¹³. The increase of globule leukocytes is in keeping with the intestinal mast cell degranulation theory.

The fact that in non-infected ν/ν mice only occasional intestinal mast cells and globule leukocytes were found is in contrast to the abundance of tissue mast cells elsewhere in the body of these athymic mice, as observed by us and others¹⁴. Furthermore, the continuous presence of the parasites⁸ did not cause an increase in either intestinal mast cell or globule leukocyte numbers.

An explanation for these findings could possibly be found in the as yet unknown origin of the mast cell. Various authors have suggested that mast cell formation may be

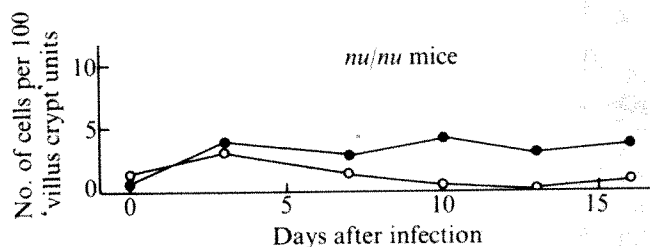


Fig. 2 Mean number of intestinal mast cells and globule leukocytes in a 10-cm portion of the proximal jejunum (expressed per 100 "villus crypt" units) in groups of 4-5 ν/ν mice infected orally with 100 *T. spiralis* larvae each. Statistical analysis (Student's *t* test). Mast cells: all days after infection against day 0, not significant. Globule leukocytes: all days after infection against day 0, not significant. \circ , Mast cells; \bullet , globule leukocytes.

thymus dependent¹⁵⁻¹⁸. Burnet¹⁶ speculated that mast cells and basophil leukocytes, or some subpopulation of such cells, are post-mitotic derivatives of thymus-derived (T) lymphocytes. On the other hand, thymic hormones can possibly also have a role in the differentiation of mast cells¹⁹.

To examine this possibility we performed reconstitution experiments. Thymus transplants of 6-week-old male donor B10LP $+/\nu$ mice were grafted subcutaneously in a group of 9 male B10LP ν/ν mice, 6 weeks of age (two thymuses per recipient). The animals were autopsied at an age of 12 weeks. A group of 10 male B10LP $+/\nu$ mice and a group of seven non-reconstituted male B10LP ν/ν mice, served as controls. Animals of both groups were 12 weeks old. The number of intestinal mast cells was counted in a 10-cm portion of the jejunum. The number of intestinal mast cells in the reconstituted mice was comparable (20-30 cells per 100 "villus crypt" units) to the number of cells counted in the jejunum of the $+/\nu$ mice, whereas none were seen in the non-reconstituted ν/ν mice which is consistent with thymus dependence of the formation of intestinal mast cells.

In all published studies results are based on tissue mast cells. In the athymic mouse tissue (non-intestinal) mast cells seem to be as abundant as in its thymus-bearing littermates. This is not consistent with the idea that the tissue mast cell is an end-stage of the T lymphocyte. In the formation of intestinal mast cells, however, the thymus clearly has an essential role.

The conclusions of the present experiments are therefore:

(1) no intestinal mast cell response accompanies a *T. spiralis*

infection in congenitally athymic (nude) mice, and (2) intestinal mast cells represent a separate population of mast cells, which is "T dependent".

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Virus-like particles and GB agent hepatitis

ALTHOUGH the MS-1¹ and CR326² strains of human hepatitis A virus have been well characterised morphologically and shown to be indistinguishable, controversy has plagued characterisation of the GB agent of hepatitis³. Presumably derived from a surgeon with acute hepatitis, the GB agent has been transmitted serially in marmoset monkeys³ but has been shown to be unrelated serologically to any known human hepatitis virus⁴. Recently, Almeida *et al.*⁵ reported the detection after prolonged search of 20-22-nm particles aggregated by endogenous antibody in a pool of marmoset sera (pool H205, GB pass 11) known to contain the GB agent. These antibody-coated particles resembled parvoviruses morphologically, appeared in "empty", "full", and fragmented crescent-shaped forms, and were undetectable in normal marmoset serum. On the basis of previous filtration data from Deinhardt *et al.*⁶ which suggest that the GB agent is approximately 20 nm in diameter and the previous finding by others that anti-complementary activity—thought to represent the presence of circulating antigen-antibody complexes—occurs during acute viral hepatitis, Almeida *et al.* suggested that the virus-like particle they have detected is the GB agent⁵.

Using a different approach in an attempt to detect the GB agent, we examined by immune electron microscopy homogenates of liver obtained during the height of acute GB infection from individual *Saguinus mystax* and *S. nigricollis* marmosets that had been inoculated with GB agent pools provided by Deinhardt. The same approach had been used successfully by Provost *et al.*² to identify the CR326 strain of human hepatitis A virus in marmoset liver. The homogenate of one liver obtained from a *S. mystax* marmoset (No. 49) (inoculated with the GB H205 pool) when serum isocitrate dehydrogenase activity was 4,680 IU (>4 times normal) was clarified and banded in a caesium chloride (CsCl) gradient (1.1-1.6 g cm⁻³). Each gradient fraction was incubated with convalescent serum from a marmoset (*S. mystax* No. 50) convalescent from hepatitis induced by the H205 pool of GB agent and examined by immune electron microscopy¹. As shown in the figure, aggregates of approximately 34-36-nm diameter particles resembling viruses with icosahedral symmetry were

detected at a buoyant density of 1.4 g cm⁻³. Both "empty" and "full" particles were observed, but empty particles were more abundant. In limited serological studies performed under code, when these particles were incubated with pre-inoculation serum from marmoset No. 50, antibody-coated aggregates were also detected, but no antibody was seen when the particle-containing gradient fraction was incubated with saline. In addition, no antibody to this particle could be detected in the convalescent serum of the surgeon from whom the GB agent was isolated.

The diameter of these particles corresponds closely to the size deduced by Parks and Melnick⁷ for the GB agent from their filtration data; however, the failure of this virus-like antigen to distinguish under code between pre-inoculation and convalescent GB serum raised questions about its relationship to the GB agent. To pursue these findings, we examined CsCl gradients from homogenates of livers obtained from five *S. mystax* marmosets with hepatitis A virus infection, from one normal *S. mystax* marmoset, from one *S. mystax* marmoset convalescent from GB infection, and from two *S. nigricollis* marmosets acutely infected with a different GB agent pool (H118).

Virus-like particles indistinguishable from those seen in

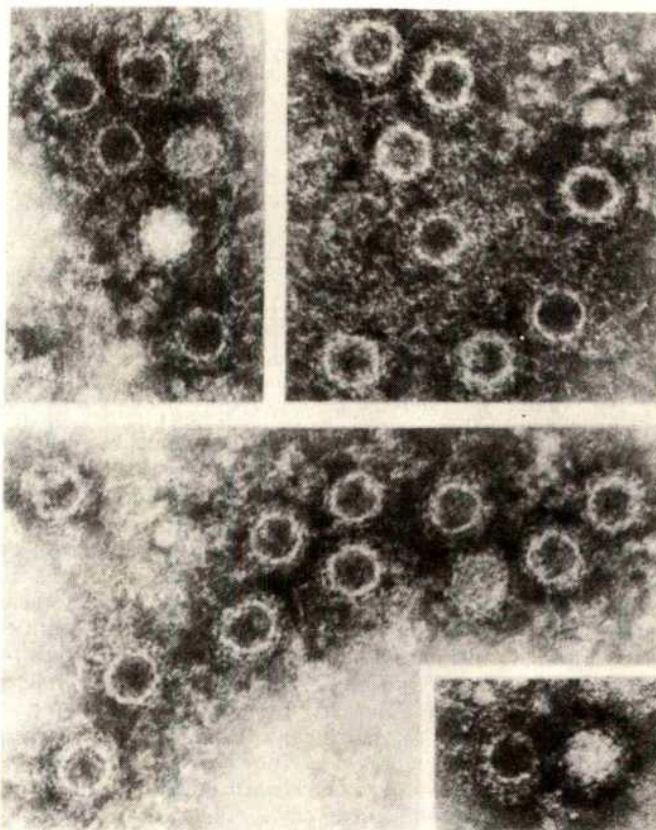


Fig. 1 Examples of aggregates of antibody-coated 34-36-nm particles detected in a liver homogenate from *S. mystax* marmoset No. 49 acutely infected with the GB agent. The liver was frozen at -70 °C immediately after the death of the marmoset. A 20% homogenate of the liver was made in distilled water after mincing with fine scissors and grinding with mortar and pestle. After the homogenate was clarified to remove large debris (1,000g for 1 h at 4 °C), 4 ml of supernatant fluid was layered on a 9-ml discontinuous preformed 6-step CsCl gradient (1.1-1.6 g cm⁻³) prepared in a cellulose nitrate tube for a Beckman SW 40 rotor (Beckman Instruments). The gradient was centrifuged in a Beckman L2-65B ultracentrifuge for 18 h at 35,000 r.p.m. (4 °C) and collected from the bottom in fractions of approximately 1 ml each. Each fraction was incubated with convalescent serum from *S. mystax* marmoset No. 50, which was convalescent from GB infection, and examined by immune electron microscopy as described¹. (Original magnification 194,400. 2% phosphotungstic acid stain.)

the first GB-infected liver were found in the two livers from marmosets acutely infected with the GB agent but in neither of the livers from the normal or GB-convalescent marmosets nor in livers from marmosets with hepatitis A virus infection (Table 1). Unfortunately, the quantity of antigenic material extracted was insufficient to allow more extensive serological evaluation, and the particles seemed to be relatively unstable after extraction.

Correlation of the size of these particles with size determinations performed by filtration of the GB agent⁷ and the specificity of their detection in GB-infected livers suggest that they may be the GB agent. If this is true, the presence of antibody in preinoculation serum is difficult to explain, unless (1) GB-related illness represents reinfection with a virus serologically related to a previously experienced virus, or (2) the GB agent is a latent virus of marmosets to which antibody is produced but which can become reactivated during periods of stress, like herpesviruses of man. Such an hypothesis agrees with the isolation of a GB-like agent from uninfected marmosets, as reported by Parks and Melnick⁸. Indeed, even if the 20–22-nm particle described by Almeida *et al.* is the GB agent, the fact that antibody to it exists in the infectious inoculum suggests an unusual immunological relationship between virus and host.

Table 1 Virus-like particles in the livers of marmosets

Category of marmoset	No. with 34–36-nm particles in liver/No. tested
GB agent-infected	
Acute	3/3
Convalescent	0/1
Hepatitis A virus-infected (acute)	0/5*
Normal	0/1

*27-nm hepatitis A antigen particles were detected in three of these.

Another discrepancy between earlier studies of the GB agent and both Almeida's and this report concerns the buoyant density of the particles. Original banding studies by Holmes *et al.*⁴ revealed a peak infectivity at a density of 1.18–1.23 g cm⁻³ for both the GB agent and the MS-1 hepatitis A virus strain. The particles identified by us have a density of approximately 1.4 g cm⁻³, and if Almeida's particles are parvoviruses or even parvovirus-like, a density of 1.4 g cm⁻³ or greater is to be anticipated. Although hepatitis A virus is known to be infectious at densities ranging from 1.15 to 1.41 g cm⁻³, the main density is thought to be approximately 1.34 g cm⁻³ (ref. 2); infectivity at densities <1.2 g cm⁻³ probably represents virions bound to lipid-containing contaminants. Conceivably the original biophysical data of Holmes *et al.* for both the GB agent and hepatitis A virus were derived with such lipid-associated material and do not represent the true density suggested by more recent studies.

If indeed, as Almeida's study suggests, the GB agent is parvovirus-like, the lipid solvent sensitivity and the instability of the GB agent reported by both Deinhardt's and Melnick's groups^{7,9} are in contrast to the marked heat stability and resistance to lipid solvents that characterise the parvoviruses¹⁰.

Clearly, data to support the candidacy of the 20–22-nm particle or the 34–36-nm particle for the elusive GB agent are incomplete. Additional studies are required to determine whether (1) either of these particles is related to GB infection, (2) an as yet unidentified particle is the GB agent, (3) GB is of human or marmoset origin, and (4) the parvovirus-like particles are related to those recently detected in human serum¹¹. Morphologically similar 22-nm particles are ubiquitous in faeces, and, although once

thought related to viral gastroenteritis, are no longer considered to be associated with any known human disease¹². Moreover, since Almeida *et al.* looked at a pool of sera, antibody from one serum might have aggregated virus in another. It will therefore be necessary to examine individual marmoset sera obtained during GB infection to establish the presence of circulating immune complexes. Until a virus-like particle is identified which can be serologically related to GB illness or can be aetiologically associated with infection by other means, morphology of the GB agent will remain a matter of conjecture.

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Avian cell transformation and the expression of avian sarcoma virus-specific tumour antigens

MALIGNANT cell transformation is often accompanied by the appearance of new cell-surface antigens^{1–3} of four classifications (defined in ref. 3): (1) embryonic antigens (EA) that are also present on normal embryonic cells; (2) differentiation antigens (DA) so called because they are also expressed on certain normal syngeneic or allogeneic cells; (3) virus structural antigens (VSA) inserted in the surface membrane of infected cells which may or may not be transformed, and (4) tumour-specific transplantation antigens (TSTA), antigens associated with tumour rejection.

Here we ask the questions first, does transformation of avian cells by agents other than avian sarcoma viruses (ASV) induce a tumour-specific surface antigen (TSSA) hitherto recognised as avian oncornavirus specific; and second, does the additional genetic information supplied by a superinfecting avian leukosis virus (ALV) or ASV lead to TSSA expression? Our experiments suggest that transformation by ASV is necessary for the induction of this antigen.

The operationally defined TSTA have received widespread attention in the past. Appropriately immunised animals will reject a subsequent challenge by viable syngeneic tumour cells because of their anti-TSTA immunity.

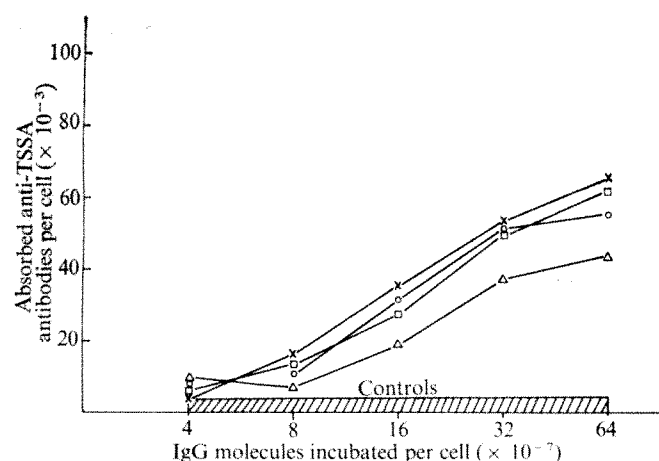


Fig. 1 Specific absorption of anti-TSSA immunoglobulins G to quail cells infected by avian leukosis sarcoma viruses and/or Kirsten sarcoma or simian sarcoma associated viruses. Cells growing in 16-mm Linbro 96 CV-TC tissue culture plates were counted and fixed at subconfluency (about 10^5 cells per culture) and incubated in duplicates with exactly equal amounts (16 μ g or dilutions thereof) of 125 I-IgG from normal or anti-TSSA (No. 62) chicken sera as described previously^{8,9}. Incubation was stopped by aspiration of the IgG dilutions and 4 washes of 5 min each with PBS. The absorbed antibodies were eluted from the cells with pH 1.2 KCl-HCl buffer and their radioactivity was assessed in a well-type scintillation counter. The number of specifically absorbed antibodies was calculated from the specific radioactivity of the anti-TSSA IgG-preparations minus background absorption of normal IgG (usually less than 20%). Quail cells were infected by the following virus strains: \times , Prague A or B77-C avian sarcoma virus strains; \square , Rous (-) sarcoma virus; \circ , 23.16 = a Rous(-) transformed quail cell line; \triangle , KiSV(SSAV) plus Prague A-ASV. Uninfected cells (controls) or cells infected by the following virus strains showed no specific absorption: RAV-1, RAV-49, KiSV (SSAV), KiSV(SSAV) + RAV-1, KiSV(SSAV) + RAV-49. All data are taken from one characteristic experiment.

Tumours induced both by chemicals and by viruses express TSTA of widely varying antigenicity and immunogenicity. Whereas in the case of chemical tumours TSTA by necessity must be cell coded, their origin in virus tumours is not known. Virus-induced TSTA, which are not incorporated into virus particles, are virus-group specific. Only virus strains belonging to the same tumour virus group will induce cross-reacting TSTAs.

In the avian oncornavirus system, a TSSA has been detected which seems invariably to be expressed on the cells of various species transformed by avian leukosis

sarcoma virus strains (reviewed in ref. 4). It is a rejection-inducing transplantation-type neoantigen (TSTA). Its origin is also unknown. It may be a direct viral gene product or appear as a consequence of virus-activated cellular genes. Chicken embryo fibroblasts infected but not transformed by avian leukosis viruses *in vitro* do not express TSSA, but chickens infected with leukosis viruses may develop immunity to tumour cell challenge, indicating that *in vivo* these viruses can elicit the antigen⁵⁻⁷. The expression of the genes coding for TSSA may in turn depend on the cell possessing a transformed phenotype and ALV may only find the appropriate target cells to form this interaction *in vivo*.

Avian cells are generally refractory to transformation by viruses other than the avian oncornaviruses. However, xenotropic oncornaviruses from mice and other mammals are infectious for avian cells. In this respect, the simian sarcoma associated virus (SSAV) is a xenotropic virus with this capability and can be used to construct a pseudotype with the Kirsten strain of murine sarcoma virus KiSV (SSAV) which will readily infect and transform Japanese quail cells. (This virus was provided by Dr R Weiss.)

Outbred Japanese quail cells between the second and tenth passage served as targets for virus infection and transformation. Only cells from a single embryo were used in any one experiment and the normal or anti-TSSA

Table 2 Uptake of radioactive 3 H-2-deoxyglucose by virus-infected quail cells

Virus	Morphology	Uptake $\times 10^{-6}$ cells (c.p.m.)	Ratio*
Uninfected	Normal	7,800	—
ALV†	Normal	9,125	1.17
ASV†	Transformed	48,830	6.26
Rous(-)-ASV	Transformed	42,275	5.42
KiSV(SSAV)	Transformed	38,455	4.93
KiSV(SSAV)	Transformed	45,865	5.88
+ ALV			
KiSV(SSAV)	Transformed	48,360	6.20
+ ASV			

*Ratio of sugar uptake of virus-infected compared with uninfected cells. Around 10^6 subconfluent cells growing in 35-mm plastic dishes were washed once in PBS at room temperature before incubation with 0.5 μ Ci ml⁻¹ 3 H-2-deoxyglucose for 9 min. After another four washes with ice-cold PBS 2 ml cold 5% TCA was added for 5 min. Two 0.5-ml aliquots were taken for assessment of radioactivity, which was adjusted to cell number determined from duplicate cultures.

† For abbreviations see Table 1.

Table 1 Growth of virus-infected quail cells in semi-solid agar*

Virus	Cell morphology	No. of cells seeded	No. of colonies	Cloning efficiency (%)
Uninfected	Normal	10^4	0	0
		5×10^4	0	0
ALV†	Normal	10^4	0	0
		5×10^4	0	0
ASV‡	Transformed	10^4	170	1.7
		5×10^4	1,900	3.8
Rous(-)-ASV	Transformed	10^4	520	5.2
		5×10^4	>4,000	>8.0
KiSV (SSAV)	Transformed	10^4	380	3.8
		5×10^4	2,300	4.6
KiSV(SSAV)	Transformed	10^4	200	2.0
+ ALV		5×10^4	1,400	2.8
KiSV(SSAV)	Transformed	10^4	650	6.5
+ ASV		5×10^4	2,700	5.4

*The agar suspension technique was performed as described in reference¹¹.

†ALV: avian leukosis virus strains used: RAV-1, RAV-49.

‡ASV: avian sarcoma virus strains used: Prague-A, B77-C.

IgG preparations were always exhaustively absorbed with normal cells before their use in the anti-globulin absorption technique^{8,9}. In this test the difference in absorption of radioactively labelled normal and anti-TSSA IgG molecules to mildly fixed target cells is taken as measurement for the amount of TSSA-expression (see legend to Fig. 1). RAV-1 and RAV-49 are avian leukosis virus strains which infect but do not transform quail cells. In contrast, the avian sarcoma virus strains Prague-A and B77-C both infect and transform quail cells as efficiently as KiSV(SSAV). The replication-defective Rous(-) strain of ASV was included in these studies for direct comparison with KiSV, which is also replication-defective in solitary infections without helper viruses like SSAV.

Since superinfection with leukosis viruses does not lead to phenotypic alterations of the infected cells, successful ALV infection of normal or KiSV(SSAV) infected quail cells had to be demonstrated by interference tests¹⁰. Dilutions of up to 10^{-7} of 1 ml of supernatant medium from each of the RAV-1 or RAV-49 infected cultures were used to infect normal quail cells and were able to prevent subsequent superinfection by focus-forming Prague-A or B77-C sarcoma virus strains, respectively.

Table 3 Phenotype and expression of the avian oncornavirus-induced TSSA of virus-infected Japanese quail cells

Virus strain	Virus type	Morphology	Growth in semi-solid agar*	Enhanced sugar uptake†	TSSA-expression‡
Uninfected		Normal	—	—	—
RAV-1, RAV-49	ALV	Normal	—	—	—
Prague-A, B77-C	ASV	Transformed	+	+	+
Rous(—)	Replication-defective ASV	Transformed	+	+	+
KiSV(SSAV)	KiSV = replication defective MuSV	Transformed	+	+	⊖
	SSAV = leukaemia-type primate oncornavirus				
KiSV(SSAV) plus ALV-strains	See above	Transformed	+	+	⊖
KiSV(SSAV) plus ASV-strains	See above	Transformed	+	+	⊕

*For details see Table 1.

†For details see Table 2.

‡For details see Fig. 1.

This high interfering titre indicated that most if not all cells had been successfully infected by ALV-strains.

Morphological transformation of quail cell cultures occurred within two passages after infection by the avian or murine sarcoma virus strains. First it had to be shown by criteria other than morphology that these cells were indeed transformed. Table 1 shows that only the sarcoma virus transformed quail fibroblasts possessed the ability to grow and form colonies in semi-solid agar¹¹. Superinfection of KiSV(SSAV) transformed cells by ALV or ASV did not alter their potential for colony formation. In contrast, uninfected or ALV-infected non-transformed cells showed no ability to grow in agar.

Another characteristic parameter of transformed cells is their enhanced rate of uptake of certain sugars^{12,13}. Again only the quail fibroblasts transformed by sarcoma virus strains expressed this property (Table 2).

Having thus defined the normal or transformed phenotype of the virus-infected cells, the specific absorption of anti-TSSA immunoglobulins to the cell surfaces was tested. As can be seen in Fig. 1, only fibroblasts infected or superinfected by avian sarcoma viruses absorbed anti-TSSA antibodies. Cells transformed by the murine sarcoma virus strain KiSV did not express TSSA even when superinfected with ALV. However, superinfection of KiSV-(SSAV) transformed quail cells with ASV strains led to TSSA-induction. All TSSA-positive cell types expressed a similar amount of TSSA, whereas the TSSA-negative cells showed practically no specific absorption.

Table 3 gives a summary of the phenotypic properties of the virus-infected cells. The encircled results point to a clear difference in TSSA expression in the cells tested.

Infection and transformation of fibroblasts by ASV leads to the induction of the avian TSSA; infection by ALV does not (reviewed in refs 2–4, and 14). TSSA-expression seems to be a consequence of ASV-transformation. All ASV-transformed cells of a variety of avian or mammalian species expressed TSSA, which is thus not only a marker for the oncogenicity of ASV, but can also be used in its partially purified form for successful anti-tumour immunisation (H. Bauer and M. Hayami, personal communication). Here we have demonstrated that ALV infection, even in a cell already possessing a transformed phenotype, does not lead to the expression of TSSA.

The sole important genetic difference in the genomes of ASV and ALV is the sarc-gene of ASV, which is not part of the ALV genome and seems to be responsible for fibroblast transformation^{15–17}. We are aware of no experimental evidence contradicting the notion that expression of the sarc-gene inevitably leads to the expression of TSSA in the plasma membrane of fibroblasts. It can, but does not necessarily mean that TSSA is coded for by the sarc-gene and thus represents a primary transforming protein. It is also possible that the primary transforming protein coded for by the sarc-gene induces TSSA synthesis by cellular genes. To distinguish between the

latter two possibilities, *in vitro* protein synthesis experiments have been initiated to attempt the transcription of the protein portion of TSSA from ASV-RNA *in vitro*.

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Immunological identification of two adenovirus 2-induced early proteins possibly involved in cell transformation

HUMAN adenovirus 2 (Ad2), a DNA tumour virus that replicates in the nucleus of permissive human cells^{1,2}, transforms non-permissive or semipermissive cultured rodent cells, but does not induce tumours in newborn hamsters (oncogenic group C). The constant functioning of protein product(s) from Ad2 'transforming gene(s)' is probably responsible for the transformed phenotype, although this has not been proved. Identification and functional characterisation of 'transforming protein(s)' are important for the understanding of cell transformation and growth control. We describe here immunoprecipitation studies made with antisera against presumptive Ad2 'transforming protein(s)', that identify two candidate transforming proteins of 53,000 (53k) and 15k daltons.

Ad2-transformed cells retain viral DNA and synthesise viral RNA sequences that are a subset of those produced during 'early' stages of productive infection, before initiation of viral DNA replication³. All Ad2-transformed cells examined contain the left 14% of the Ad2 genome, that is map positions (MP) 0–0.14 (ref. 4). These cells express mRNA from Ad2 DNA about MP 0.02 to 0.10 assuming a continuous transcript, that are also expressed

early⁵. Some Ad2-transformed cells contain and express other sections of the viral genome, but none contain the entire genome^{4,5}. Cultured rat cells are transformed by transfection with G fragment (MP 0-0.075) generated by cleavage of Ad2 or Ad5 (oncogenic group C) DNA with restriction endonuclease *HindIII*, and digestion of more than 1% of DNA by exonuclease III + *S*₁ nuclease results in loss of transforming activity⁶. These studies indicate that information for cell transformation lies somewhere between Ad2 MP 0.01 and 0.075, and is expressed early after infection.

To identify Ad2 transforming protein(s) we prepared antisera against viral proteins expressed in Ad2-transformed rat cells by injecting sonicated F17 cells into rats. Cell line F17 (P. H. Gallimore) contains only the left 14% of the Ad2 genome⁴, and thus viral proteins should include and be mainly transforming proteins. We also used antisera from hamsters immunised with extracts of tumours induced in hamsters by Ad2-SV40 and Ad1-SV40 (Ad1 is in oncogenic group C) hybrid viruses (antisera were obtained from Dr R. Gilden⁷). The Ad2 DNA sequences present in the Ad1- and Ad2-SV40-induced tumours have not been determined. IgGs from F17 and hamster antisera were used to immunoprecipitate ³⁵S-labelled polypeptides produced in human KB cells early after productive infection. Polypeptides were identified by polyacrylamide slab gel electrophoresis and

autoradiography. Injection of Ad2-transformed rat or hamster cell extracts into rats or hamsters, respectively, should minimise induction of antibodies to rat or hamster proteins, and therefore decrease the danger of immunoprecipitation of human proteins from Ad2-infected KB cells by possible cross-reacting antibodies to rat or hamster proteins. Accordingly, proteins specifically immunoprecipitated by F17, Ad1-, or Ad2-SV40 IgG from Ad2-infected KB cell extracts should be virus specific.

In studies of Ad2 early proteins cells are treated with arabinosyl cytosine (ara C) 4h after infection and labelled in the presence of ara C; this prevents viral DNA replication and consequently the onset of late stages of infection, so that only early viral polypeptides are labelled. Two polypeptides (75k DNA binding protein, 21k) are in infected cell extracts (Fig. 1b), that are absent from uninfected cells (Fig. 1a). Treatment of cells with cycloheximide before labelling enhances viral protein synthesis relative to host⁸. In this gel 5 Ad2 induced early proteins are visible after cycloheximide pretreatment (Fig. 1d): 75k, 21k, 19k, 15k, and 11k (a 11.5k polypeptide forms part of the 11k band). Using longer gels (27cm) with higher resolution we have observed as many as 13 Ad2-induced early polypeptides, but do not know whether each is a product of a unique viral gene.

Immunoprecipitation studies using F17 IgG are shown in Fig. 1, e-l. Cells were not pretreated with cycloheximide, and were

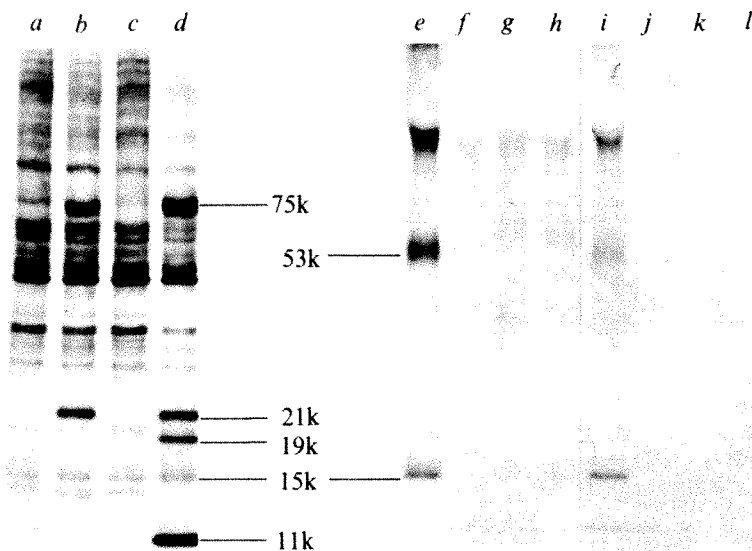
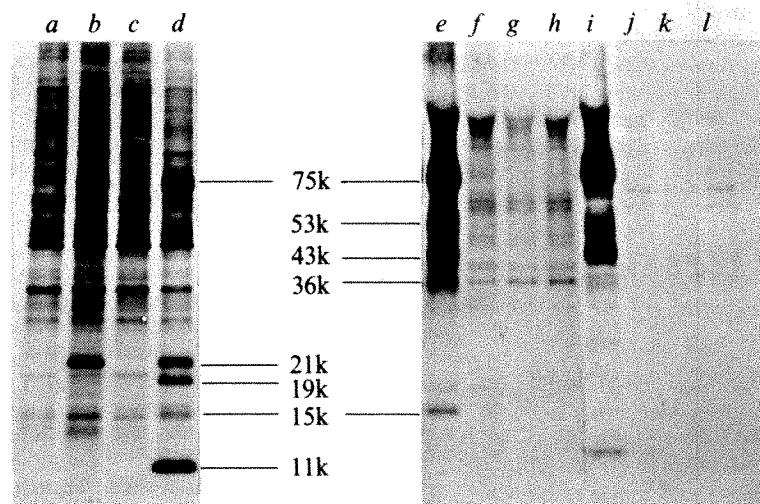


Fig. 1 Autoradiogram of sodium lauryl sulphate-polyacrylamide slab gel showing electrophoretic separation of ³⁵S-polypeptides induced in human KB cells by Ad2 early after infection, and immunoprecipitated by rat antiserum against extracts of Ad2-transformed F17 rat cells. KB cells suspended in Eagle's MEM at 6×10^6 cells per ml were infected with 100 plaque-forming units per cell of Ad2^{9,10}. A second culture of KB cells was treated in a similar manner in this and subsequent steps, but without addition of virus, to serve as a mock-infected control. After 1h adsorption cells were diluted to 3×10^5 cells per ml with MEM containing 5% horse serum and 10 mM HEPES, pH 7.2. Four hours after infection arabinosyl cytosine (ara C) was added to $20 \mu\text{g ml}^{-1}$. At 6h after infection cells were resuspended at 9×10^5 cells per ml in methionine-free MEM containing 5% horse serum, 10 mM HEPES, pH 7.2, and ara C ($20 \mu\text{g ml}^{-1}$), and labelled for 6h with ³⁵S-methionine ($25 \mu\text{Ci ml}^{-1}$, 273 Ci mmol⁻¹). After labelling, cells were washed twice by centrifugation with ice-cold phosphate-buffered saline (PBS), suspended at 18×10^6 cells per ml in reticulocyte standard buffer, allowed to swell for 15 min, and disrupted by 30 strokes of a tight-fitting Dounce homogeniser. Nuclei were collected by centrifugation and the perinuclear membrane was removed by the 'double-detergent' washing procedure¹⁴ using Tween 40 and sodium deoxycholate. Nuclei were suspended in radioimmune precipitation (RIPA) buffer¹⁵, sonicated for 3 min, incubated at 20°C for 150 min, and clarified by centrifugation (4°C) at 31,000g for 20 min. RIPA buffer is 10 mM sodium phosphate, pH 7.2, 150 mM NaCl, 1%, Triton X-100, 1%, sodium deoxycholate and 0.1% sodium lauryl sulphate. The postnuclear cytoplasm was mixed with 0.1 volume of $10 \times$ RIPA buffer, incubated at 20°C for 15 min, and clarified as described for nucleoplasm. F17 cells for immunisation were grown in Ca^{2+} -free MEM supplemented with 10% foetal calf serum. Before each injection, cells were collected by scraping with a rubber policeman, washed three times in PBS, and sonicated for 3 min. A sonicated 10% cell suspension in PBS was emulsified with 1 volume of Freund's complete adjuvant and 0.5 ml of emulsion was injected intraperitoneally into each rat. Eight injections were performed at weekly intervals and rats were exsanguinated 9d after the last injection. Immune sera from four Sprague-Dawley and three Wistar rats were pooled. IgG was isolated from the F17 serum pool (F17 IgG) or from a pool of normal rat sera (non-immune IgG) by standard methods as previously described^{9,10}. Samples from the infected or mock infected cytoplasmic (9×10^6 c.p.m.) or nuclear (3×10^6 c.p.m.) extracts were mixed with $30 \mu\text{g}$ of F17 IgG or non-immune rat IgG in 150- μl reaction mixtures containing RIPA buffer. Reaction mixtures were incubated in 0.4-ml plastic disposable centrifuge tubes (Beckman) for 18h at 4°C . Goat serum anti-rat IgG ($28 \mu\text{l}$) was added and the incubation continued for 2h at 37°C . The contents of the tubes were layered on 180 μl sucrose (10% sucrose in RIPA buffer) and centrifuged for 1.5 min at 10,000g in a Beckman 152 microfuge. The supernatants were removed by suction and the precipitates were washed three times by vigorous mixing with 150 μl of RIPA buffer, then resuspended in 20 μl of sample buffer⁸ without 2-mercaptoethanol. Radioactivity in an aliquot of each sample was determined. An average of 0.26% of input counts was in control precipitates (infected cytoplasmic extracts against non-immune IgG, or mock infected cytoplasmic extracts against both non-immune and F17 IgG) whereas 0.69% of input counts were in precipitate from infected cytoplasmic extract against F17 IgG. The precipitates of the corresponding controls of the nuclear extracts contained an average of 0.64% of input counts, whereas the precipitate from infected nuclear extract against F17 contained 1.66% of input counts. Equal volumes (7 μl) from each precipitate suspension were boiled for 4 min and analysed by sodium lauryl sulphate polyacrylamide gel electrophoresis in 9-10-cm gradient slab gels (8-21%) as previously described⁸ except that slabs were 0.75 mm thick. Extracts of mock-infected and Ad2-infected cells were also included to serve as markers. These were obtained as described above except that cells were labelled with ³⁵S-methionine ($25 \mu\text{Ci ml}^{-1}$) for 1h only (7.5-8.5h after infection). Two other cultures, infected and mock infected, were prepared by the cycloheximide enhancement method⁸. Briefly, cycloheximide ($25 \mu\text{g ml}^{-1}$) was added 1h and ara C ($20 \mu\text{g ml}^{-1}$) at 4h after infection. At 7h after infection cells were washed twice with warm medium containing ara C ($20 \mu\text{g ml}^{-1}$), and labelled for 1h with ³⁵S-methionine as described above. The cells were collected, washed with ice-cold PBS, precipitated with 10% trichloroacetic acid, solubilised in sample buffer, and electrophoresed on polyacrylamide gels as before⁸. After electrophoresis, gels were dehydrated in a solution of dimethyl sulphoxide, soaked in a solution of 2,5-diphenyloxazole in dimethyl sulphoxide¹⁶, dried, exposed to film at -70°C , and autoradiographs were developed. a, Uninfected cells, ara C-treated; b, infected cells, ara C-treated; c, uninfected cells, CH- and ara C-treated; d, infected cells, CH- and ara C-treated; e, infected cytoplasmic extract against F17 IgG; f, infected cytoplasmic extract against non-immune rat IgG; g, uninfected cytoplasmic extract against F17 IgG; h, uninfected cytoplasmic extract against non-immune rat IgG; i, infected nuclear extract against F17 IgG; j, infected nuclear extract against non-immune rat IgG; k, uninfected nuclear extract against F17 IgG; l, uninfected nuclear extract against non-immune rat IgG.

fractionated into cytoplasm (*e-h*) and nucleoplasm (*i-l*). F17 IgG specifically precipitated 51–55k (average 53k) and 15k polypeptides from both cytoplasmic (Fig. 1*e*) and nuclear (Fig. 1*i*) extracts of infected cells. IgG from unimmunised rats (non-immune rat IgG) did not precipitate these polypeptides from infected cell cytoplasmic (Fig. 1*f*) or nuclear (Fig. 1*j*) extracts. Neither F17 nor non-immune rat IgG precipitated 53k and 15k polypeptides from uninfected cell extracts (Fig. 1*g* and *k*, and *h* and *l*, respectively). Several minor host bands were precipitated nonspecifically by all IgG samples. The swelling at the top of the lanes of Fig. 1 representing immunoprecipitated samples is caused

polypeptides⁸. We have observed, however, a 53k polypeptide in infected cells by labelling in hypertonic medium (unpublished results), which greatly reduces the synthesis of host proteins relative to viral proteins. Thus both 53k and 15k polypeptides can be detected, in special labelling conditions, that could correspond to the immunoprecipitated polypeptides. We have also observed 53k and 15k in various protein labelling and immunoprecipitation experiments: (1) with and without pretreatment of cells with cycloheximide; (2) with cells labelled between 5 and 8h, 7 and 7.5h, 8 and 11h (but not 2 and 5h), 6 and 10h followed by a 12-h chase, and both with and without 2 mM phenyl-

Fig. 2 Autoradiogram of polyacrylamide slab gel, showing electrophoretic separation of polypeptides precipitated by Ad1-SV40 IgG from cytoplasmic and nuclear extracts of Ad2 early infected KB cells. The same markers and extracts described in the legend to Fig. 1 were used, and precipitated in the same manner, except that 20 μ g of Ad1-SV40 IgG or non-immune hamster IgG was used. Goat serum anti-hamster IgG (10 μ l) was added after 18h of incubation at 4°C. Fraction of input counts recovered in precipitates: cytoplasmic controls, average 0.23%; infected cytoplasmic extract against Ad1-SV40 IgG, 1.58%; nuclear controls, average 0.61%; infected nuclear extract against Ad1-SV40 IgG, 3.99%. Placement of the lanes is identical to that described in Fig. 1 except that Ad1-SV40 IgG replaced F17 IgG and non-immune hamster IgG replaced non-immune rat IgG.



by IgG (150k). In this experiment samples were not treated with 2-mercaptoethanol to reduce disulphide bonds as this dissociates IgG into 50k and 25k subunits that distort the lower gel portions. Mercaptoethanol treatment did not affect the 53k and 15k bands, nor reveal any additional virus-specific polypeptide bands. The protein band immediately below the IgG in Fig. 1*e* and *i* was not present when mercaptoethanol was used, and therefore probably is an artefact of antigen-antibody dissociation or electrophoresis, and not a high molecular weight virus induced polypeptide.

As Fig. 2 shows, Ad1-SV40 IgG also specifically immunoprecipitated 53k and 15k polypeptides from the cytoplasm of infected cells (Fig. 2*e*), and 53k but not 15k from the nucleoplasm (Fig. 2*i*). The Ad1-SV40 titre to 15k seems to be lower than that of F17, since it precipitated less 15k from the cytoplasm, and apparently none from the nucleus. Neither 53k nor 15k were precipitated from uninfected cell extracts by Ad1-SV40 IgG (Fig. 2*g* and *k*), or from infected (Fig. 2*f* and *j*) or uninfected (Fig. 2*h* and *l*) cell extracts by non-immune hamster IgG. Ad1-SV40 IgG also precipitated large amounts of 75k, as expected from our earlier studies with Ad1- and Ad2-SV40 antisera proving that 75k is expressed in the Ad1- and Ad2-SV40-induced hamster tumours^{9,10}. Also precipitated were diffuse bands of 43k and 36k polypeptides (Fig. 2*e* and *i*), which are degradation products of 75k (ref. 11 and unpublished data). Ad2-SV40 IgG gave identical results (data not shown). Saborio and Öberg¹² reported that Ad2-SV40 IgG precipitated 72k, 67k, 60k, and 45k polypeptides from extracts of labelled Ad2-infected HeLa cells. How these correspond to the polypeptides we have observed is not known. It is possible that they did not detect our 15k polypeptide because of the low titre of Ad2-SV40 IgG against this polypeptide.

Unequivocal identification of the 53k and 15k polypeptides as virus-specific is difficult by radioimmune precipitation and gel electrophoresis, because of possible cross-reacting antibodies, nonspecific precipitation and proteolytic breakdown of immunologically reactive viral proteins. At 15k polypeptide is visible in extracts without immunoprecipitation, especially after cycloheximide pretreatment, but a 53k polypeptide usually cannot be resolved from the large background of host

methylsulphonylfluoride (protease inhibitor); (3) with two independent pools of F17 antisera; (4) with and without absorption of F17 antiserum with extracts of uninfected KB cells. In these various experiments 53k was observed as a fairly diffuse band as in Figs 1 and 2. Some variability was observed in the polypeptides in the 15k region of gels. Occasionally a polypeptide of about 17k was observed in cytoplasmic extracts (Fig. 1*e*), and one of 11k in nuclear extracts. The 11k polypeptide apparently induced by Ad2 and localised in the cell nucleus (unpublished), was also precipitated by non-immune IgG, probably nonspecifically. Also the 15k polypeptide band sometimes appeared as a doublet and even as a triplet. The significance of this variability is unclear: the 'extra' bands may represent some type of modification of 15k. It is also possible that F17 and the Ad-SV40 antisera contain low titres of antibodies against viral polypeptides of 15–17k in addition to 15k.

Our major finding is that three antisera (F17, Ad1-SV40 and Ad2-SV40) expected to contain antibodies against Ad2 transforming protein(s) specifically precipitate 53k and 15k polypeptides. The synthesis of both polypeptides in Ad2-transformed rat cells, Ad-SV40 hamster tumour cells, and Ad2-infected human cells, suggests (but does not prove) that they are virus coded. Since F17 contains only the left 14% of the Ad2 genome⁴, which includes transforming genes, these polypeptides are strong candidates for transforming proteins. It is important to note that F17 cells apparently express Ad2 mRNA sequences in addition to those of presumptive transforming genes; that is Ad2 transforming genes map somewhere within MP 0.01 and 0.075 (ref. 6), whereas F17 may express roughly MP 0.03–0.10 as mRNA⁵. It is therefore possible that either 53k or 15k (or both) is not a transforming protein. The only evidence available on this point is from Lewis *et al.*¹³, who have translated *in vitro* Ad2 mRNA, hybridisation-purified from F17 cells, into a diffuse 44–50k and a prominent 15k polypeptide. The 15k polypeptide was produced by translation of mRNA selected by hybridisation to either *HindIII*-G (MP 0–0.075) or *HindIII*-C (MP 0.075–0.17), but not *HpaI*-E (MP 0–0.041). As *HindIII*-G can transform cells this argues that the 15k polypeptide may not be a transforming protein, unless there is more than one 15k, or unless the 3'-

terminal sequences (complementary to *HindIII*-C) in the mRNA for the 15k polypeptide are superfluous. The 44–50k polypeptide mapped to the left of 15k and thus its gene is closer to the transforming region. Of course it is not known whether our 53k and 15k correspond to the 44–50k and 15k polypeptides of Lewis *et al.*, but if so, then 53k may be a better candidate than 15k as a transforming protein. Positive identification of Ad2-transforming protein(s) will require transformation-defective mutants, as well as antisera against transformed cells that express only transforming gene(s).

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Growth regulation of cells grown in suspension culture

VARIOUS aspects of a cell's growth cycle can be manipulated in tissue culture. For example, the initiation of DNA synthesis and, in some cases, culture growth, can be enhanced by serum¹ and platelet² components, polypeptides purified from growth-conditioned medium³ and urine⁴, insulin⁵, corticosteroids⁶, prostaglandins⁷, cyclic nucleotides (see, for example, ref. 8) and proteolytic enzymes⁹. In addition, at least three purified proteins can initiate DNA synthesis in fibroblast-like cells—nerve growth factor¹⁰, epidermal growth factor¹¹ and fibroblast growth factor^{12,13}. Disturbance of the culture medium directly over the cultured cells also induces DNA synthesis¹⁴. Fibroblast-like cells have been used in most of these studies, and they grow tightly associated with the culture dish and are strongly anchorage dependent for division^{15,16}. Given the strong anchorage dependence of the test cells, two alternatives could account for the induction of DNA synthesis and growth by the diversity of conditions and "factors" outlined above. The first would ascribe the effects of these compounds to interactions on the cell surface with a suitable receptor. These are hormone-like interactions, thought to be exemplified by compounds such as insulin and purified

growth factors¹. The second alternative represents interactions which function directly through alterations of cell anchorage. For example, the growth stimulating effects of the proteases are presumably affected by modification of the cell-substratum (anchorage) interaction¹⁷. To distinguish between these two alternatives, it should be sufficient to assay the effects of serum and a purified growth factor on the initiation of DNA synthesis in an anchorage-dependent cell line, and on a variant which grows in suspension culture. If serum and the growth factor were equally effective in both cell lines, then a disturbance in anchorage could not account for the mitogenic activity, ruling out the second alternative. The first, hormone-like, mechanism, however, would be excluded if the growth factor was unable to induce DNA synthesis in the cells grown in suspension. The following experiments support this latter possibility by showing that fibroblast growth factor^{12,13} does not affect the growth of myoblast cells grown in suspension culture.

The cells examined were a rat myoblast line (L6) derived from embryonic skeletal muscle¹⁸ and a subline (M3A) derived from it which grows in suspension culture¹⁹. Although M3A does not fuse to form myotubes, its growth characteristics and regulation of some muscle enzymes are the same as the attached parent cell line¹⁹. When tested in several concentrations of serum, the serum dependence for growth of M3A and L6 cultures is similar, with the M3A cells responding slightly less well to low serum concentrations (Fig. 1). Finally, 3T3A, an anchorage-dependent line whose response to various growth factors has been well defined²⁰, was used as a control to test the efficiency of the purified growth factor.

A growth promoting factor for fibroblast cells, initially found in pituitary extracts¹² and subsequently purified¹³, was used because it seems to initiate DNA synthesis in a wide variety of cell types¹³. This factor, termed fibroblast growth factor (FGF), was purified²¹ and used in the following studies. The assay for the initiation of DNA synthesis was done on sparse quiescent cells²⁰. Table 1 shows that purified FGF stimulated the incorporation of ³H-thymidine by resting 3T3 cells as described before²⁰, demonstrating that the FGF preparation was functional on the fibroblasts.

When attached L6 myoblasts were tested for stimulation of DNA synthesis by FGF in conditions identical to those described for 3T3, there was an increased incorporation of ³H-thymidine into trichloroacetic acid (TCA)-precipitable material (Table 1). The greatest stimulation was with FGF

Table 1 Effect of FGF on fibroblasts, anchorage-dependent myoblasts and suspension grown myoblasts

Additions	Cell lines and relative increase in thymidine incorporation		
	3T3A	L6	M3A
None	1	1	1
10% Serum	17.1	7.1	5.8
FGF	4.5	1.4	1.1
Insulin	—	2.0	1.0
Hydrocortisone	1.0	1.1	0.9
Hydrocortisone + insulin	3.3	2.1	1.1
FGF + insulin + hydrocortisone	6.6	7.0	1.2

Cells were plated in 10% foetal calf serum, and after 2 d the serum concentration was reduced to 0.5%. 48 h later the indicated reagents were added to the growth-inhibited cells (density between 5×10^4 and 2×10^5 cells per 60-mm dish). 18 h later the incorporation of ³H-thymidine into TCA-precipitable material was assayed as described²⁰. The concentrations of the reagents (ng l⁻¹) were: FGF, 100; insulin, 1,000; hydrocortisone, 1,000. Data are presented as the increase, relative to control cultures in 0.5% serum, of the ³H-thymidine incorporation. Background incorporation in control cultures was 5,100 c.p.m. for 3T3A, 6,800 c.p.m. for L6 and 7,900 c.p.m. for M3A. The data are the means of triplicate determinations, with an average error between samples of less than 10%. The experiment was repeated three times with similar results.

in combination with insulin and hydrocortisone. But when the suspension-grown cell line, M3A, was assayed in the same conditions there was little or no stimulation of ^3H -thymidine incorporation with the defined reagents (Table 1), although the stimulation achieved by the addition of whole serum was similar to that found with L6. Thus the combination of FGF, insulin and hydrocortisone, compounds which act synergistically with FGF to stimulate DNA synthesis 3T3 cells²⁰, stimulate anchorage-dependent L6 cells but not their suspension-grown variant. Since both L6 and its anchorage-independent derivative M3A are responsive to serum stimulation to approximately the same extent (Fig. 1 and Table 1), the possibility that FGF and its synergists can replace the serum requirement of these cells is ruled out. In addition, if M3A had lost its hypothetical surface receptors for FGF, then FGF has no rate limiting physiological role in the growth regulation of these cells by serum.

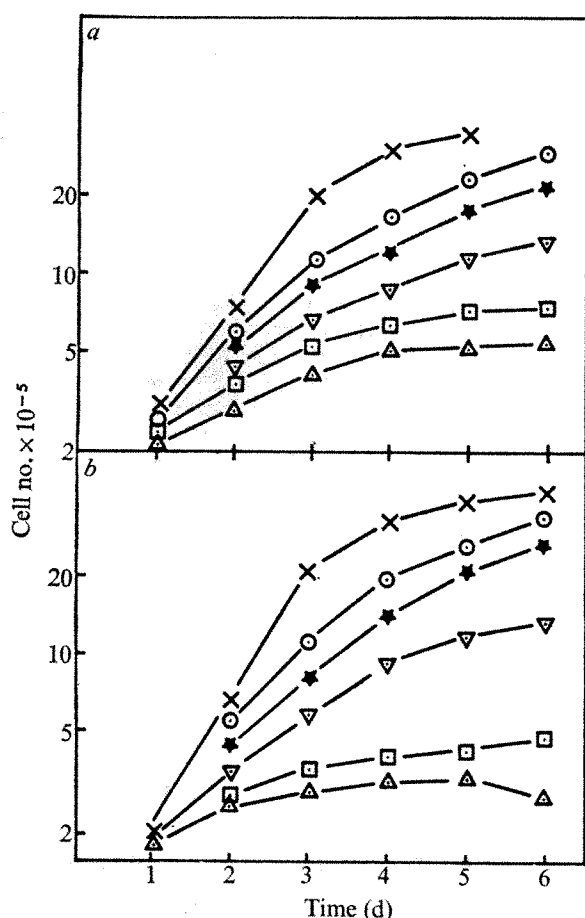


Fig. 1 Serum dependence for culture growth of L6 and M3A cells. Cells were plated on day 0 in 60-mm tissue culture (L6) or Petri dishes (M3A) in 5 ml of medium containing the indicated concentration of foetal calf serum. The cell number was then followed as a function of time. *a*, L6; *b*, M3A; Δ , 0.2% serum; \square , 0.5% serum; ∇ , 1% serum; \star , 2% serum; \times , 10% serum.

If it is generally true that cells grown in suspension culture are not sensitive to the defined "factors" which stimulate the growth of attached, anchorage-dependent cells, then some caution should be exercised in interpreting results based exclusively on attached cells. Of the conditions that tend to stimulate growth of attached fibroblast-like cells, there seems to be one underlying factor—the disturbance of the limiting cell membrane. For example, insulin stimulates growth^{5,20} and is weakly proteolytic²²; the corticosteroids are lipophilic and interact nonspecifically with membranes. Nerve growth factor stimulates the growth of fibroblast-like cells, presumably through its proteolytic subunit¹⁰, as do

various proteolytic enzymes^{9,17,23}. Since overt proteolytic treatment of anchorage-dependent cells inhibits division by rendering the cells into suspension, it follows that the extent of proteolysis that initiates cell division is quantitatively less. In addition, disturbance of the medium above cells triggers DNA synthesis¹⁴, perhaps perturbing the cell-substratum interactions by exerting a weak shear force on the cells. Various inorganic ions are nonspecifically mitogenic²⁴, perhaps for the same reason. As the degree of cell anchorage is critical in the initiation of cell division (see, for example ref. 17), it is possible that any reagent which disturbs this cell surface interaction in attached cells may trigger one or more rounds of DNA synthesis and cell division. Such a reagent need not be proteolytic. For example, several purified proteins lacking proteolytic activity induce neurite retraction in C1300 neuroblastoma cells, presumably by weakening the cell-substratum interaction required for neurite extension²⁵.

In summary, if purified (serum) growth factors are functioning in a hormone-like manner by interacting with specific cell surface receptors, then one would predict that anchorage-dependent and independent lines should respond similarly if their serum response is the same. As this hypothesis is contradicted by the data reported here, it is possible that "growth factor" responses of attached cells are not mediated through specific surface receptors, but through less specific perturbations of the cell-substratum interaction. For anchorage-dependent cell lines, anchorage is necessary but not sufficient to regulate cell division. By using anchorage-independent cells for the characterisation of growth promoting activities, it is possible to eliminate one experimental variable.

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Effect of 3-acetylpyridine on tissue differentiation of the embryonic chick limb

3-ACETILPYRIDINE (3-AP) and 6-aminonicotinamide have a teratogenic effect on the development of the chick limb but have no effect of coadministered with nicotinamide¹. This

has led Caplan to the hypothesis that nicotinamide has a crucial role in the spatial organisation of muscle and cartilage in the developing limb, high levels of nicotinamide specifying muscle, and low levels cartilage; with even the possibility that a metabolic gradient related to the blood supply could provide the positional information specifying the pattern of muscle and cartilage²⁻⁵. The evidence for this hypothesis is based both on *in vivo* and *in vitro* studies, the latter claiming to show that 3-AP enhances chondrogenesis and inhibits myogenesis in cultured limb mesenchyme cells. As regards the *in vivo* effects, it is suggested that the gross muscle hypoplasia observed might be accounted for in terms of 3-AP switching the differentiation of mesenchyme cells from a myogenic fate to a chondrogenic one. This seemed to us to be an unlikely explanation, since the chondrogenic and myogenic tissues are already physically separated at stages when 3-AP is still known to have an effect (as late as 13 d of incubation). It seemed much more likely that 3-AP was affecting muscle growth rather than its differentiation. We therefore made a histological study of the effect of 3-AP, and found that it was a potent destroyer of the peripheral nerves, and that there is no evidence to support the view that it affects mesenchyme differentiation.

Eighty-four White Leghorn embryos at 3 d of incubation were windowed and sealed with Sellotape. At 6 d of incubation, 2 mg of 3-AP (Sigma) in 100 μ l of distilled water were dropped on to the embryo membranes of 56 of these, the remainder serving as controls. The eggs were resealed and incubated for periods from 6 h to 5 d when the embryos were removed and the right wings and legs fixed in Karnovsky's solution and embedded in Araldite. Transverse sections (1 μ m) were taken from a region of the leg 1.5 mm proximally from the distal end of the tibia, where the thin appearance of treated limbs is most marked, and from the wing mid-way along the radius and ulna, limbs being measured and marked in Araldite before cutting. The sections were stained with Toluidine blue, and areas measured from camera lucida drawings with a Stanley Planimeter. By 3 d after treatment, the usual gross morphological appearance of 3-AP treatment (described as 'muscle hypoplasia' and evident particularly as thinness of the legs) was discernible, and by 5 d it was marked, with embryos being

reduced in wet weight by up to 30% and their legs, although of approximately normal length, reduced by up to 50% in thickness. As is usual in both teratogenic and genetic defects, the legs were affected much more markedly than the wings, and there was considerable variation in expression of the defects, ranging from almost normal appearance to death.

At 6 d of incubation, when treatment began, the cartilage of both wing and leg is well differentiated but ossification has not yet begun. The dorsal and ventral muscle masses are clearly visible, and the splitting division into individual muscles is just beginning to be evident, but the tendons have not yet begun to develop. The physical separation of chondrogenic from myogenic tissues should be stressed, the cartilage being surrounded by a well defined perichondrium. The nerves at this stage extend as far as the wrist and ankle. Sections were taken from five legs which had been left 5 d after treatment, together with five equivalent controls (Fig. 1). Notable is the absence of tendons, and the reduction in the area of the cartilage of the treated animal (49% reduction in the average area of the tibia). It is these factors, together with the reduction in the loose mesenchyme which give the limb its characteristic thin appearance at this stage; there is in fact virtually no muscle normally present in this region so the usual description of 'muscle hypoplasia' is inappropriate. Further, nerves could not be found in the treated legs, although they were well developed in the controls.

This last result has been noted previously by Tanaka *et al.*⁶, although they remained uncertain as to its exact importance. The effect is apparent on peripheral rather than on spinal nerves, although the neurotoxic effect of 3-AP on adult brains has been noted by several authors⁷.

Since 3-AP is described as specifically affecting differentiation of the muscles, sections were taken from the muscle-rich region of the wing described above. Sixteen treated wings at various stages were examined, together with 12 controls. The muscle up to 2-3 d after treatment was substantially normal, though somewhat smaller than the controls; and the muscle blocks began to split up, again as normal, giving rise to individual muscles. After 3 d the muscles began to degenerate. There was no significant

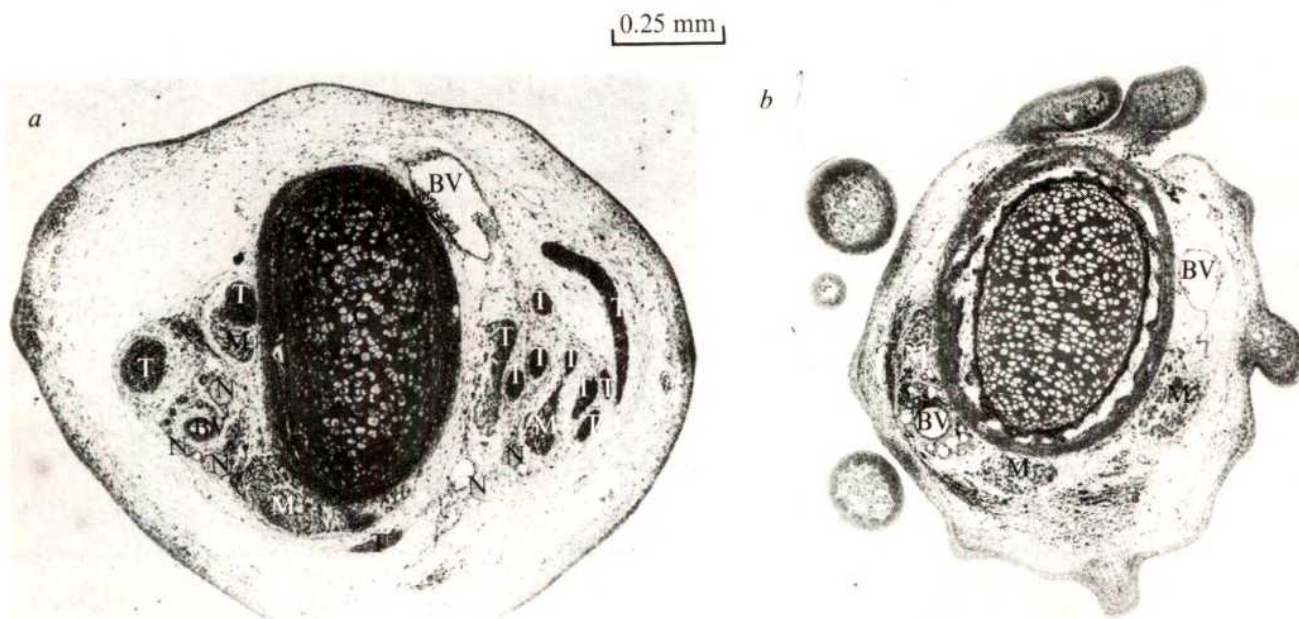


Fig. 1 C, Cartilage; M, muscle; T, tendon; N, nerve; BV, blood vessel. *a*, Transverse section of leg of 11-d-old control animal. The cartilage element is the tibia. *b*, Transverse section of leg of animal treated with 3-AP at 6 d, 5 d after treatment. Tendons and nerves are absent and the cartilage is smaller in area.

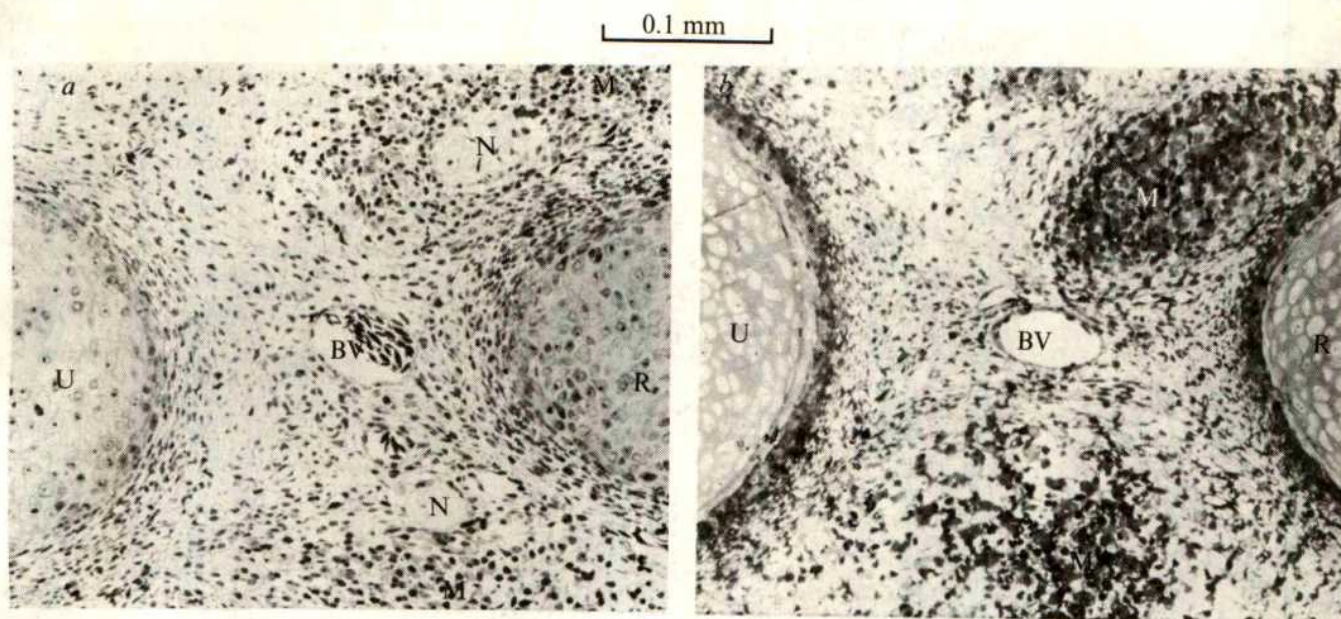


Fig. 2 R, Radius; U, ulna; N, nerve; M, muscle; BV, blood vessel. *a*, Enlargement of central region of transverse section of normal 6-d-old chick wing (that is, at time of treatment). Nerves are clearly present. *b*, Same region of limb treated with 3-AP, 24 h after treatment (that is, at 7 d of incubation). The nerves present at 6 d have now been destroyed, but the muscle blocks are not obviously affected.

reduction in the area of the cartilage; the most striking effect was on the nerves. In embryos examined 6–12 h after treatment, some nerves were already absent. Examination at 24 h or later showed all limbs except one to be completely devoid of nerves (Fig. 2).

These results show that 3-AP has a deleterious effect on the growth of all the tissues in the developing limb, that on nerves being the most marked. Since it is well known that denervation results in muscle degeneration^{8,9}, the long term effect of 3-AP on muscles is most probably mediated by its effect of destroying peripheral nerves within about 24 h of treatment. The absence of tendons may also have an adverse effect on muscle development. 3-AP can also reduce the radial growth of cartilage viewed in cross section, although the length of the cartilaginous elements is not affected, perhaps because of interference with cell division, leaving matrix secretion unaffected. Conversely, our preliminary results suggest that 6-AN does affect matrix secretion. The greater effect on the leg when compared with the wing, can be related to the comparatively small amount of radial growth in the radius and ulna during the experimental period compared with the tibia (twofold as opposed to ninefold for the latter).

The effect of 3-AP on the tissues of the limb bud as revealed by histology shows clearly that it does not act specifically by repressing myogenesis and enhancing chondrogenesis in the manner proposed by Caplan, but rather indicates a deleterious effect on various cell types including cartilage, mesenchyme, muscle and tendon, but which is particularly marked in the case of peripheral nerves, these being almost totally destroyed after only 24 h treatment, and whose absence must lead to serious defects in the later development of the muscles. The biochemical mechanisms involved in the specification of muscle and cartilage remain quite unknown.

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Development of the locust ocellus

WORKERS interested in retinal development have been attracted by the iterative unit structure and prolonged development time of the compound eye of hemimetabolic insects^{1–4}. By comparison, the ocellus of the adult locust (*Schistocerca gregaria*) is a simple retina composed of only 1,000 receptive elements with a very loose cartridge-like division of receptors and underlying synaptic plexus. The plexus is composed of receptor terminals, the arborisations of second-order cells and glial elements. Most of the second-order contributions to this structure are composed of the repeatedly branched endings of the so-called giant cells, on to which the retina is highly convergent. The structure is so simple that here I report the possibility of examining developmental mechanisms at the single-cell level.

In eggs maintained at $28^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ the ocelli first become visible through the embryonic cuticle as small white patches about 178 h before emergence (mean total development time 380 h). Sections cut at this stage show the ocelli as cone-shaped aggregations of 'tailed' epidermal cells. No distinct growth cones are present and few neurotubules are found in the cytoplasm. By 165 h before emergence the epidermal cells have extended 60–70 axonal processes $225\text{ }\mu\text{m}$ long, deep into the brain. This gives the embryonic retinula axons a growth rate of about $17\text{ }\mu\text{m h}^{-1}$, somewhat slower than the $25\text{ }\mu\text{m h}^{-1}$ recorded for *Lucilia* compound eye retinula cells⁵. The axons are rich in neurotubules and the cytoplasm contains an abundance of ribosomes and granules.

On entering the embryonic neuropile the axons establish conical growth cones rich in filopods. During growth to this point growth cones are typically spear shaped. Within the brain the filopods of the retinula cells make electron dense contact with certain neuropilar processes. At this

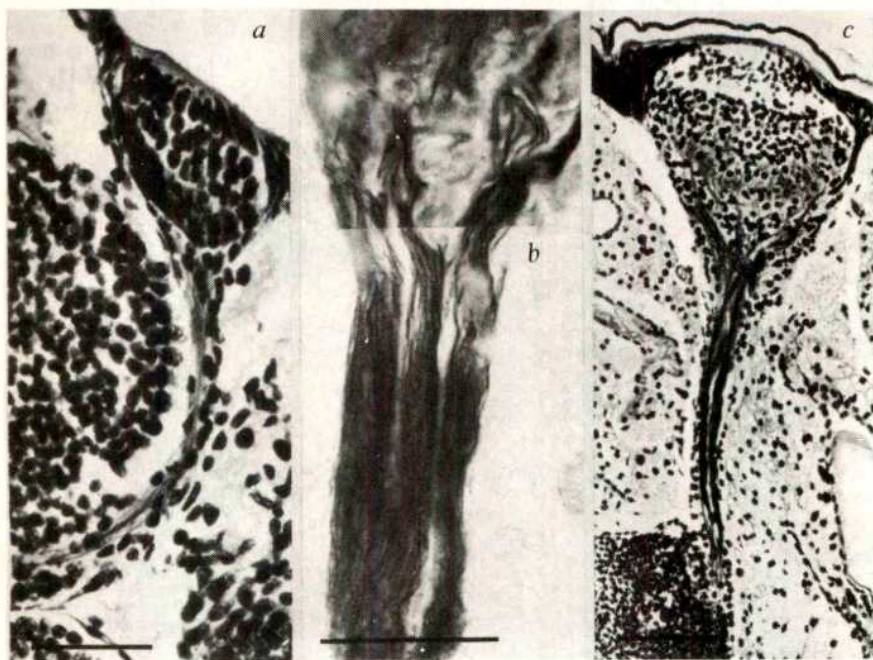


Fig. 1 *a*, A 10- μ m wax section, stained by the Power-Bodian technique⁷, of the lateral ocellus of a newly emerged hopper. The ocellar nerve containing about 70 retinula cell axons skirts the protocerebral lobe and enters the protocerebrum near the midline. Most such axons terminate in the dorsal pars intermedia. Many of these axons are derived from rhabdomeless cells and thus cannot be functional at this stage. The bar represents 50 μ m. *b*, The retinal axons of the newly emerged hopper appear tightly grouped around the second-order axons, only three of which lie within this section. The bar represents 25 μ m. *c*, The lateral ocellus of a second instar locust during the intermolt period. Note the vast expansion in the number of cells in the retina; retinula cell axons are absent from the length of the ocellar nerve. Branches pass from the second-order cells into the descending retinal bundles. The darkly stained group of cells (top left) are thought to represent the next group of retinula cells to be recruited from the surrounding epiderm. The bar represents 100 μ m.

stage no second-order processes can be detected in the descending bundle of retinula cell axons.

Sections taken shortly before emergence (380 h) show the presence of five large second-order cell profiles in the

ocellar end of the lateral ocellar nerve. The second-order profiles are readily distinguished from the receptor terminals because of their white cytoplasm. Two hours after emergence the retina cell axons still extend within the brain but only to the top of the protocerebral lobe (Fig. 1*a, b*). This positional change is not due to any change in length of the retinula cells but rather to a sustained growth of the second-order axons and an associated increase in the length of the ocellar nerve. Synapse-like structures are present in the ocellar nerve 6 h before emergence but usually in association with dense-cored vesicles.

After emergence into the light, synaptic structures proliferate rapidly. The lateral ocellar second-order axons extend in the course of the first two instars until all the retinula cell axons lie within the proximal half of the ocellar nerve (Fig. 1*c*). New retinula cells are recruited from the surrounding epiderm at each moult, and their axons are incorporated into the synaptic plexus.

Examination of silver-stained, semi-thin and thin sections shows increase in the complexity of the second-order giant cell arborisations with each moult, retinula cell ingrowth apparently preceding the addition of branches to the giant cells. The presence of two axons connecting the median ocellus with the lateral ocellus^{5,6} makes possible the staining of these two cells' arborisations by the diffusion of cobalt chloride into the median nerve (Fig. 2*a*). In the adult these two arborisations consist of repeated branching into the paths of the descending retinula cells. Stains of earlier instars reveal a much reduced branching structure. It is interesting to speculate that the apparent contact guidance of second-order cells from the brain into the ocellus may be followed by the advance of long collaterals from the second-order cells into retinula cell bundles through a similar mechanism in which the newly developed retinula axons compete for collaterals. Support for this theory comes from adult brains that occasionally contain a third supernumerary median to lateral cell^{5,6}. In this circumstance the arborisations of each individual cell are simpler than in the normally innervated retina (Fig. 2*a* and *b*). Manipulative experiments are in progress to confirm these observations and to determine what other parameters may affect the form of the second-order cell

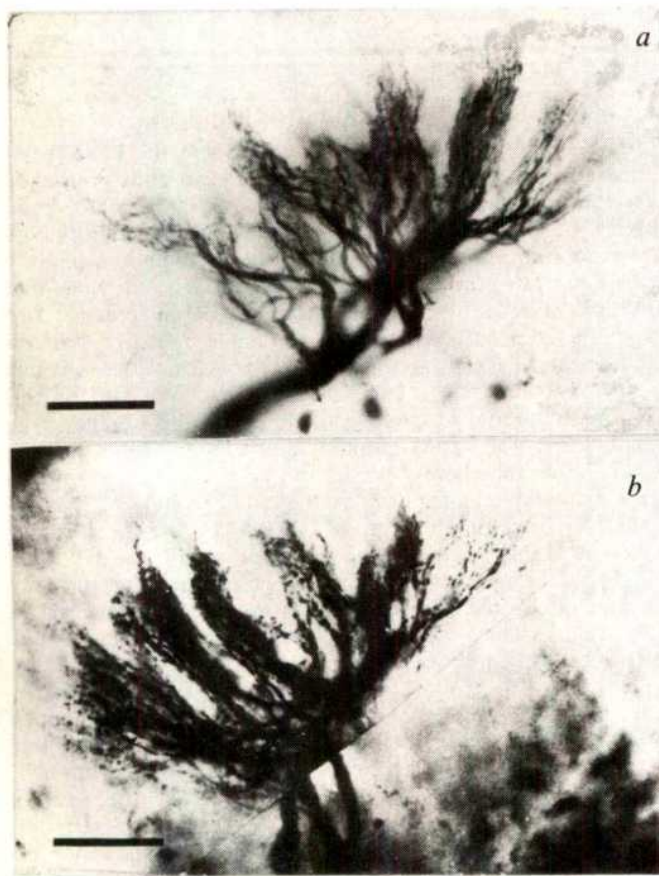


Fig. 2 *a*, Cobalt stain of the two latero-median axons viewed as a whole mount of the adult lateral ocellus. *b*, Whole mount of an adult lateral ocellus which contains a third latero-median axon. In spite of the presence of an extra nerve there is no commensurate increase in the total number of collaterals generated. The bars represent 100 μ m.

patterns. Further structural details of ocellar development are to be discussed elsewhere.

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Plasmodium falciparum gametocytogenesis *in vitro*

THE mechanism of sexual reproduction among malaria parasites is coming under increasing scrutiny. Gametogenesis is being unravelled by electron microscopy¹ and by kinetic studies². Gametocytogenesis on the other hand remains poorly understood in spite of earlier work^{3–5}. The development of *Plasmodium falciparum* gametocytes is particularly intriguing because in man they develop in the deep tissue spaces, especially the spleen and bone marrow. The immature stages only rarely appear in the peripheral blood and as a result have escaped detailed experimental investigation until now. Furthermore, it has long been suspected that their development is prolonged³, taking 8–12 d, although there are suggestions to the contrary^{6,7}. Immature gametocytes of *P. falciparum* have recently been reported in cultures thought to be composed only of asexual parasites⁸. I have used a similar microculture technique which has permitted the development *in vitro* of morphologically mature *P. falciparum* gametocytes and present here some preliminary observations on the process of gametocytogenesis in *P. falciparum* infections.

Parasites were obtained from 27 unselected patients aged 6 months–6 yr. Parasitaemias were measured by standard techniques. Heparinised venous blood was washed once with

medium 199. From each patient cultures were set up composed of medium 199 supplemented with 200 mg% glucose, 30 mg% glutamine, 25% (v/v) foetal calf serum and 2.5% (v/v) of washed packed blood cells. Aliquots (200 μ l) of the culture were dispensed into flat-bottomed 6-mm tissue culture microplates (Linbro) and maintained in a CO₂ incubator (5% CO₂ in air) at 37 °C. The medium was replaced every 2 d. The culture was sampled each day by taking one 200- μ l aliquot for the preparation of blood smears.

Table 2. Development *in vitro* of gametocytes from parasites in samples of blood taken from patients on succeeding days

Patient	Days on which blood was taken	Asexual parasites present in blood taken from patient (per 10 ⁵ red cells)	Immature gametocytes grown <i>in vitro</i> from the blood samples (per 10 ⁵ red cells)
AJ	0	231	26
	2	610	222
YD	0	480	20
	2	44	5
JG	0	577	2
	1	4,318	1
	2	1,095	2
	3	3,100	6
MG	0	2,727	3
	1	2,040	13
	2	238	1
	3	40	6
	6	154	5
	10	1,194	275
	14	1,926	53

Gametocytes developed in 80% of cultures; that is in 22 taken from 27 different subjects. The cultured gametocytes were classified into stages of development⁹ based on the detailed morphological descriptions made by earlier workers from post-mortem specimens and tissue biopsies^{4,10}. As a result a sequential pattern of development for the growing gametocytes was demonstrated (Fig. 1). The significance and relevance of this morphological pattern to the developmental biology of *P. falciparum* gametocytes is under study. In man the gametocytes of *P. falciparum* first appear in the peripheral blood about 10 d after the asexual parasites^{3–5}. The cultures *in vitro* give direct confirmation that the 10 d are occupied by the progressive development of the gametocytes rather than by an inductive process and a shorter period of development⁷. The mean culture life was 8.8 d during which all the immature gametocytes grew to at

Table 1 Growth and multiplication of trophozoites, and the development of gametocytes of *Plasmodium falciparum* *in vitro*

Patient	Time (h) since culture started	No. of parasites per 10 ⁵ red cells	Rings	Small trophozoites	Large trophozoites	Early schizonts	Mature schizonts	Gametocytes* I II III	Dead parasites (asexual and sexual)
SB	0	1,450	72	28	—	—	—	—	—
	24	1,210	—	14	12	26	12	36	—
	48	5,190	62	29	—	2	1	3	—
	72	4,650	21	39	7	—	—	4	13
FC	0	900	100	—	—	—	—	—	—
	24	850	8	24	26	18	—	14	—
	48	3,520	30	52	4	1	1	30	16
	72	4,364	16	64	7	2	—	30	34
MG	0	1,926	40	40	20	—	—	—	—
	24	1,889	—	18	60	20	—	2	—
	48	5,632	40	22	8	8	16	4	2
	72	7,302	18	50	16	6	—	—	4
AJ	0	610	40	50	10	—	—	—	—
	24	880	6	22	22	36	6	8	—
	48	2,700	40	44	—	13	2	1	—
	72	4,760	22	32	21	8	—	6	5
	96	4,770	9	9	25	4	—	5	45

*Gametocytes classified according to Hawking *et al.*⁹ (see Fig. 1).

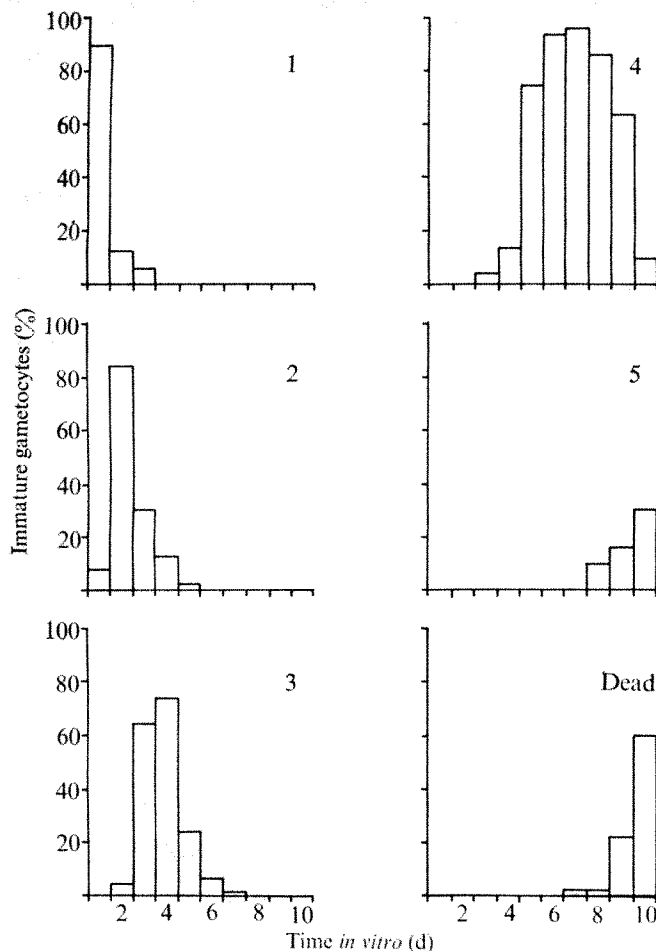


Fig. 1 Sequential development of immature gametocytes of *Plasmodium falciparum* grown *in vitro*. The gametocytes were classified into stages of development according to the outline of Field and Shute⁴ and modified by Hawking *et al.*⁹; 5 developmental stages were recognised. (1) Rounded parasite, not filling the red cell; (2) parasite irregular shape, slightly elongated, not filling the red cell; (3) parasite elongated, red cell slightly distorted; (4) parasite elongated, red cell very distorted; (5) mature crescentic gametocyte. Dead gametocytes were identified as parasites within red cells that were either elongate and very densely stained or were rounded up and lightly stained. These probably represent gametocytes that died at different stages of their development.

least the 4th stage of development and some cultures produced up to 32% of morphologically mature gametocytes. There is known to be an interval of 1–2 d between the development of morphological maturity and the attainment of functional maturity¹¹. It seems that the longevity of the cultures, limited so far by the ability of red cells to survive prolonged periods *in vitro* at 37 °C has prohibited the development *in vitro* of functionally mature gametocytes.

The asexual parasites in the original sample of blood grew *in vitro* and after 36–48 h significant reinvasion of erythrocytes by the liberated merozoites was obtained (mean multiplication rate of 4.8 times). During the first 24 h *in vitro* no increase in parasite numbers was recorded and yet immature gametocytes were detected (Table 1). It is clear therefore that very young gametocytes, as newly formed rings, invade the peripheral blood along with the young asexual rings that come from the same population of schizonts. Both populations then withdraw to the deep tissues to complete their development, the asexual parasites taking a further 18–24 h and the gametocytes 8–9 d. Phillips *et al.*⁸ have further reported that ring-stage parasites derived from the peripheral blood and cultured *in vitro* to the schizont stage can subsequently give rise to both sexual and asexual lines of *P. falciparum* parasites *in vitro*.

Gametocytogenesis in *P. falciparum* infections within

adult humans has been positively correlated with the onset of clinical malaria rather than simply with increasing asexual infection⁷. The event is probably not therefore the consequence of an intrinsic process that gives an increasing probability of detecting gametocytes from a higher compared to a lower asexual infection. Only 59% of my cultures started from patients with asexual parasites alone in their peripheral blood produced developing gametocytes *in vitro*, compared with 100% of the cultures taken from patients with an infection that had already started to produce mature gametocytes *in vivo*. These results confirm that gametocytes are not produced immediately the blood infection begins, rather there is a definite point in the infection when gametocytogenesis starts for the first time. Once gametocyte production has begun from the persisting asexual infection, however, then each successive schizogony produces further gametocytes. Four patients studied on more than one occasion supported this view (Table 2). It is suggested that there is a trigger for the start of gametocytogenesis but once the process is established within an infection then gametocyte production becomes an intrinsic part of the life cycle.

The culture technique described has opened a new area of the life cycle of *P. falciparum* to detailed investigation. The preliminary observations confirm the prolonged development of *P. falciparum* gametocytes. They also show that once gametocyte production has begun each schizogony is responsible for fostering sexual reproduction the elements of which make a temporary appearance in the peripheral blood before withdrawing to the deep tissues.

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Cell surface design

CONSIDERATIONS of membrane fluidity (see refs 1–5) have often involved schematic representations of the cell plasma membrane and underlying cytoskeletal structures^{3–5}. But, although intended to show how microtubules, microfilaments and so on might interfere with the mobility of membrane components, such models have not been drawn to scale. Usually microtubules and microfilaments seem much smaller than the plasma membrane, whereas the reverse is true. Thus, it has not been possible to evaluate fully the relative importance of plasma membrane and cytoskeleton in influencing cell surface dynamics. I have therefore tried to draw these various elements to scale. Since the data come from heterogeneous sources (fibroblasts, muscle cells, amoeba, plant cells, epithelial cells and lymphocytes) it is clear that the organisation of the various structures represented here can only be hypothetical and does not correspond to a known structure in a given cell type.

Figure 1 is a representation of such a structure; it shows a section of microvillus (MV), with actin microfilaments (MF), α -actinin molecules (α -A), myosin molecules arranged as a filament (MM); the cortical cytoplasm with microtubules (MT) and their cross bridges (c-b), the plasma-membrane (PM) with extrinsic and intrinsic proteins (drawn

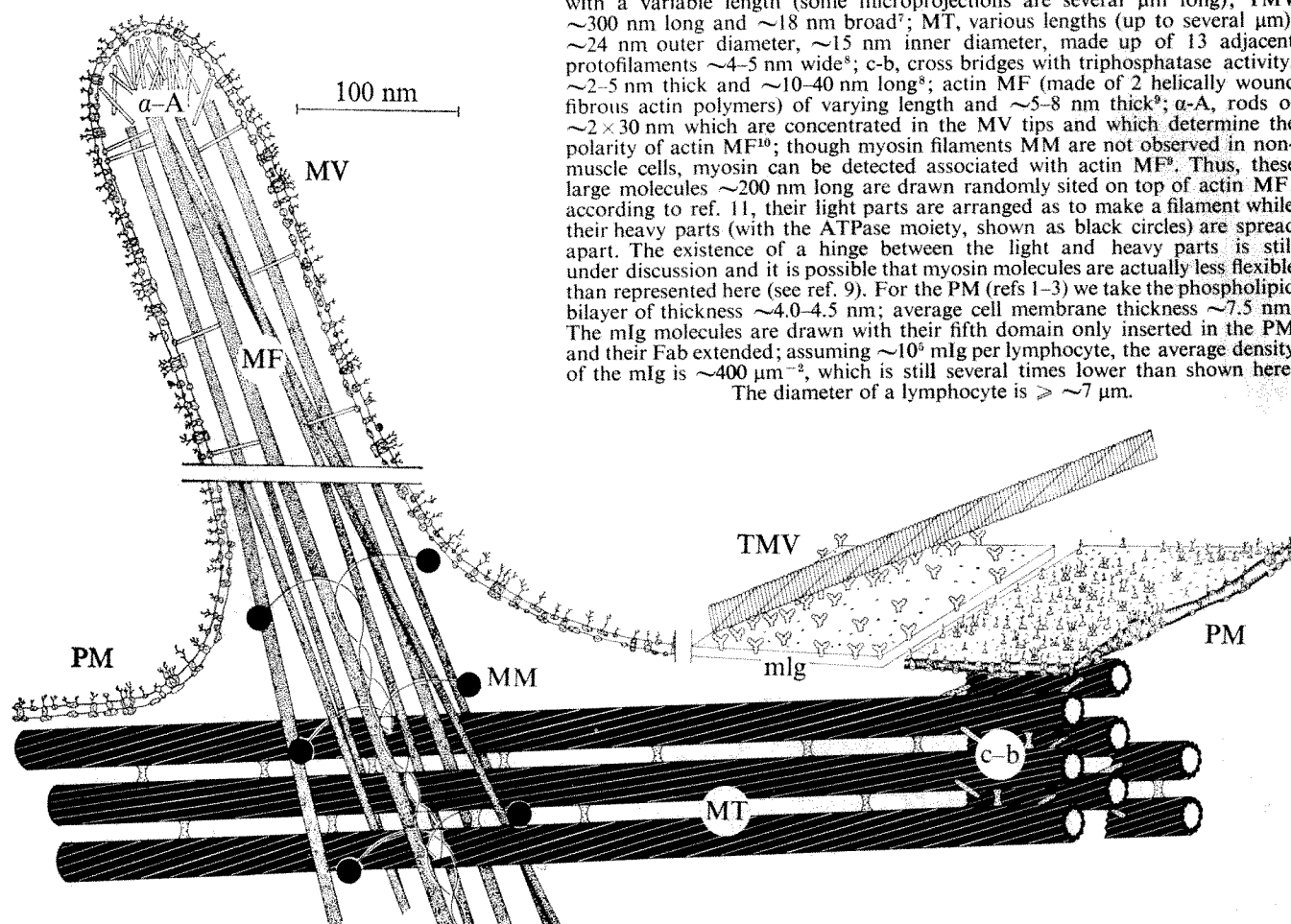


Fig. 1 Schematic representation of the cell surface; the enlargement can be estimated from the size of the tobacco mosaic virus particle (TMV) adsorbed on some mlg molecules. Actual sizes are as follows: MV diameter ~ 100 – 200 nm with a variable length (some microprojections are several μ m long); TMV ~ 300 nm long and ~ 18 nm broad⁷; MT, various lengths (up to several μ m), ~ 24 nm outer diameter, ~ 15 nm inner diameter, made up of 13 adjacent protofilaments ~ 4 – 5 nm wide⁸; c-b, cross bridges with triphosphatase activity, ~ 2 – 5 nm thick and ~ 10 – 40 nm long⁹; actin MF (made of 2 helically wound fibrous actin polymers) of varying length and ~ 5 – 8 nm thick⁹; α -A, rods of $\sim 2 \times 30$ nm which are concentrated in the MV tips and which determine the polarity of actin MF¹⁰; though myosin filaments MM are not observed in non-muscle cells, myosin can be detected associated with actin MF⁹. Thus, these large molecules ~ 200 nm long are drawn randomly sited on top of actin MF; according to ref. 11, their light parts are arranged as to make a filament while their heavy parts (with the ATPase moiety, shown as black circles) are spread apart. The existence of a hinge between the light and heavy parts is still under discussion and it is possible that myosin molecules are actually less flexible than represented here (see ref. 9). For the PM (refs 1–3) we take the phospholipid bilayer of thickness ~ 4.0 – 4.5 nm; average cell membrane thickness ~ 7.5 nm. The mlg molecules are drawn with their fifth domain only inserted in the PM and their Fab extended; assuming $\sim 10^6$ mlg per lymphocyte, the average density of the mlg is $\sim 400 \mu\text{m}^{-2}$, which is still several times lower than shown here. The diameter of a lymphocyte is $\geq \sim 7 \mu\text{m}$.

to the typical Singer–Nicolson pattern) and with an area which shows some receptor immunoglobulins (mlg) and belongs to a lymphocyte surface (for a complete analysis, see ref. 6).

This model is very different from all those so far published. For instance, it now seems impossible that several microfilaments or microtubules could be linked to single plasma-membrane proteins, as has usually been postulated. On the contrary, several membrane proteins might be linked to single microfilaments or microtubules, by means of elements such as α -actinin molecules or microtubule cross bridges. Furthermore, it seems unlikely that phospholipid flow is the major force driving the redistribution of membrane components in processes such as 'capping', on lymphocytes as well as on other cells. More likely, contractile microfilaments are involved in such gross cell surface rearrangements, the principal role of microtubules being more static. I have suggested elsewhere⁶ an hypothesis of how the antagonistic functions of microfilaments and microtubules might be co-ordinated. My idea is that they would confer to the cortical cytoplasm of the cell properties like those of a thixotropic gel.

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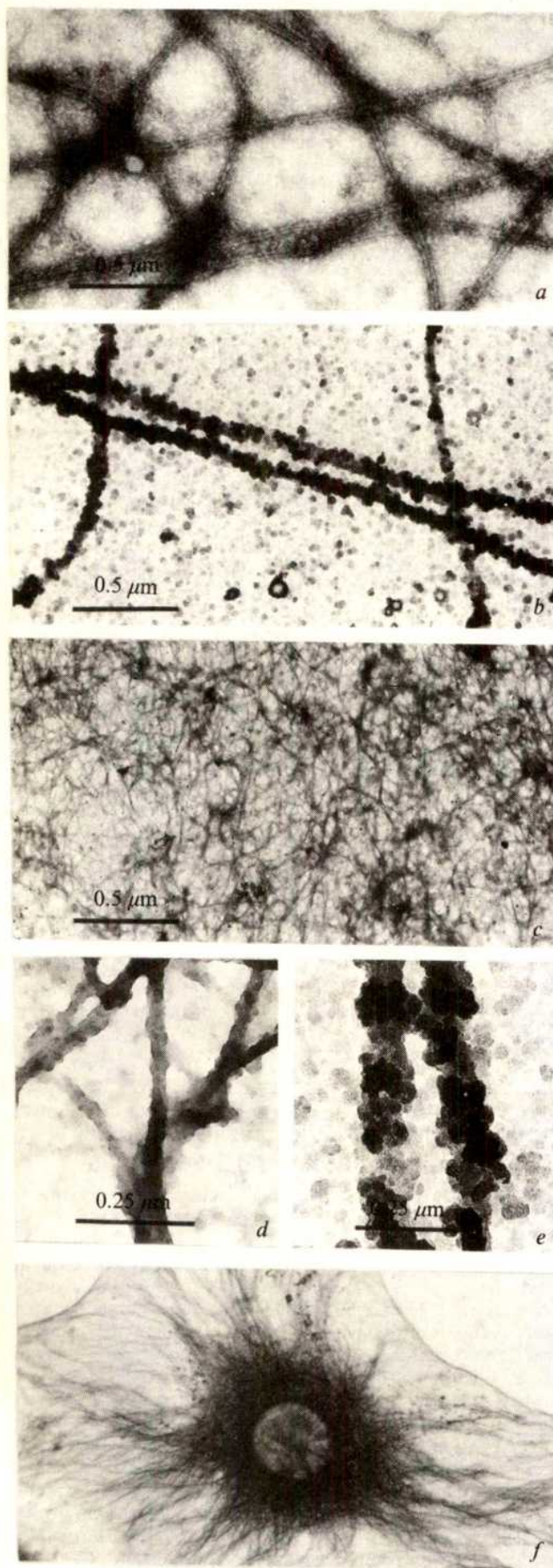
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Immunoperoxidase visualisation of microtubules and microtubular proteins

MICROTUBULES are ubiquitous eukaryotic organelles, formed by polymerised tubulin and presumably some additional proteins, which are involved in a wide variety of cell functions (for review see refs 1 and 2). Evidence has been presented for the existence of a dynamic equilibrium between polymerised and non-polymerised microtubular proteins³. Recently immunofluorescence of cultured cells demonstrated a fine cytoplasmic network that was believed to correspond to cytoplasmic microtubules. However, no direct evidence was presented that the antibodies stain individual microtubules, the formaldehyde fixation that was used is not known to preserve microtubules intact and immunofluorescence does facilitate comparison of the total picture of the microtubular apparatus with its appearance in histological and ultrastructural sections (horizontally or vertically) through the same cell. Moreover, the inadequate resolution of the light microscope does not allow an accurate localisation of the



non-polymerised tubulin and the study of its interaction with various cellular organelles. We have therefore developed an immunochemical technique described here that could simultaneously be used for the localisation of microtubules and tubulin in whole cells and in histological and ultrastructural sections, after adequate fixation with glutaraldehyde.

The unlabelled peroxidase-anti-peroxidase (PAP) method of Sternberger⁶ which had previously been adapted for the localisation of neurophysins⁷ seemed to fulfil the necessary criteria. In this method, the antibody associated with tubulin is allowed to react with an excess of an anti-immunoglobulin (as in the fluorescent "sandwich" method), and then, in turn, with a soluble complex of peroxidase and its antibody. The latter complex acts as an antigen and combines with the free antibody sites and may then be revealed by chemical reactions that lead to the deposition of osmium.

Before attempting to use whole cells, we have assessed whether microtubules polymerised in a cell-free system can be stained specifically with this method.

Figure 1b shows that individual microtubules polymerised *in vitro* and fixed with glutaraldehyde can, indeed, be stained by the PAP method. In the electron microscope

Fig. 1 *a*, Microtubules polymerised *in vitro* and negatively stained with uranyl acetate. Tubulin was prepared from rat brain homogenate and polymerised as described previously⁸. One drop of the solution was placed on a nickel grid coated with Formvar. After 1 min the grid was rinsed with a few drops of hexylene glycol 1 M and fixed for 1 min in 2.5% glutaraldehyde at room temperature. After rinsing with sodium cacodylate 0.1 M the grids were placed for 30 s on a drop of 0.5% uranyl acetate, then drained and dried. *b*, Microtubules polymerised *in vitro* and stained with the PAP-technique. Nickel grids with microtubules obtained and fixed as described above were placed on a drop of normal goat serum diluted 1/30 in Tris-buffered saline (TBS), pH 7.4 for 10 min. Then the grids were washed for 10 min by flotation on TBS on a rocking table. Hereafter the grids were placed for 30 min on a drop of TBS containing a 1/3,200 dilution of antibody solution II (see Table 1) and 1% goat serum. After washing in TBS for 20 min the grids were placed for 30 min on a drop of goat antiserum to rabbit immunoglobulins (1/20 in TBS) (GAR/Ig Nordic Pharmaceuticals and Diagnostics), and subsequently washed with TBS. Thereafter the grids were placed for 30 min on a drop of TBS containing a 1/50 dilution of the PAP complex and 1% goat serum, and subsequently washed. This was followed by the reaction with 3,3'-diaminobenzidine (DAB) for 7 min, staining with osmium tetroxide 2% on 0.05 M veronal acetate, washing with veronal acetate and drying. The reaction with DAB was carried out according to Weir *et al.*¹¹. Note the accumulation of darkly stained PAP complexes on the microtubules and the diffuse layer of PAP complexes between them. *c*, Actin filaments polymerised *in vitro* according to the method of Carsten¹². The fibres were adsorbed on to nickel grids covered with Formvar, fixed with glutaraldehyde and stained with the PAP method using the antibody dilution, in an identical fashion as described for *b*. Note the complete absence of PAP complexes from the filaments. *d*, Microtubules polymerised *in vitro* and stained with the PAP method as described above with omission of the antibody solution II. Note the complete absence of PAP complexes from the microtubules and from the spaces between them. The enlargement of the microtubules (± 35 nm instead of ± 24 nm) is probably due to the rehydration and the coating with normal goat serum. *e*, Part of *b* enlarged to the same magnification as *f*. Untransformed mouse embryonal cell in culture (MO)¹³ stained with the PAP-technique. Cells, grown on cover slips, were fixed in 0.25% glutaraldehyde in 0.1 M sodium cacodylate pH 7.4, for 5 min. After washing in the same buffer (30 min), they were postfixed with a series of acetone 50% \rightarrow 100% \rightarrow 50% at 4°C; and placed in TBS for 15 min; brief washing in TBS; 1/800 antibody dilution (no. II, Table 1) in TBS \times 1% normal goat serum, 30 min; extensive washing as before; 1/20 GAR, 30 min; extensive washing as before; 1/300 PAP in TBS containing 1% normal goat serum, 30 min; extensive washing in TBS. The reaction with DAB was the same as in Fig. 1b. The reaction product was stained with osmium tetroxide (2% in veronal acetate) for 10 min at 4°C. After washing, the cover slips were mounted in Fluormount with the cells downwards. Note the dense cytoplasmic network and the absence of nuclear staining, except for pronounced staining of the nuclear membrane ($\times 500$).

individual microtubules appear as heavily stained arrays of PAP complexes with a diameter of ± 100 nm. This size can be expected in view of the diameter of microtubules (± 24 nm), and of the PAP complex (± 20 nm) linked to the microtubules by a double layer of immunoglobulins. Because of this enlargement the labelled microtubules are clearly visible when the grids are examined with the light microscope. Fixation with formaldehyde (3.5%; 5 min) results in a distortion of the microtubules and a loss of the protofilamentous substructure at the ultrastructural level. But these structures still bind the immune complex. After glutaraldehyde or formaldehyde fixation PAP complexes are evenly distributed in the open spaces between the microtubules (Fig. 1b). The staining pattern is identical when microtubules are polymerised directly from a 100,000g supernatant from homogenised rat or rabbit brain⁸ or when microtubules are prepared from a tubulin solution that is enriched by two polymerisation-depolymerisation steps⁹.

Table 1 Results obtained with different antibody solutions and different substrates

Substrate	Antibody solution	Dilution	Staining
Microtubules	II purified γ -G	1/800–1/50,000	+
	Immune rabbit serum	1/100,000	—
Microtubules	I γ -G	1/800–1/6,400	+
	Immune rabbit serum	1/12,500	—
Actin	I	1/3,200	—
Collagen	I	1/3,200	—
Microtubules	III	1/800–1/3,200	+
	Antiserum against purified tubulin		
Microtubules	IV RAB/Alb	1/800	—
Microtubules	V adsorbed γ -G normal rabbit serum	1/800	—

Antibody solution I was prepared by injecting rat brain tubulin, purified by two polymerisation-depolymerisation steps⁹, homogenised with Freund's complete adjuvant. The immunoglobulin serum fraction was obtained by two precipitations with sodium sulphate. The protein concentration was 36.6 mg ml⁻¹. Antibody solution II was derived from solution I through further purification by affinity chromatography on Sepharose-4B to which the antigen was coupled. The protein concentration was 5 mg ml⁻¹. Antibody solution III is a crude antiserum raised against SDS-gel purified tubulin. Antibody solution IV is a rabbit anti-bovine serum albumin antiserum (RAB/Alb Nordic Pharmaceuticals and Diagnostics, Antwerp, Belgium). Antibody solution V is an immunoglobulin G fraction from normal rabbit serum adsorbed on to a column containing Sepharose-4B to which tubulin was coupled. The protein concentration was 15 mg ml⁻¹.

When rabbit muscle actin filaments, or rat skin collagen fibres¹⁰, polymerised *in vitro*, are subjected to an identical procedure, they remain completely unstained (Fig. 1c). Neither is any background visible, as is the case with formvar-coated grids that were not covered with any protein. Microtubule staining is also completely absent if one or more of the immune reagents is omitted (Fig. 1d). When rabbit anti-bovine serum albumin IgG or purified rabbit γ -globulins, previously absorbed on to a column of Sepharose 4B to which tubulin was coupled, are used the microtubules are not stained. Neither is any background staining visible. The results that were obtained with various antibody solutions on different substrates are given in Table 1.

Preliminary results, using the fixation procedure of Brinkley *et al.*⁴ but the PAP method instead of immunofluorescence showed that a fine cytoplasmic network and the mitotic spindle can be visualised in cultured cells at the light microscopic level. This resembles the immunofluorescent picture in all respects. Moreover, a similar network is visible after fixation with glutaraldehyde (Fig. 1f). The network seems to consist of more individual lines that have a greater definition than after formaldehyde fixation. Finally, semithin

(1 μ m) and thin sections (0.07 μ m) can be made of the same cells after embedding them in Epon.

In semithin sections the cytoplasmic network is visible either sectioned longitudinally or transversely.

At the ultrastructural level, both cytoplasmic and mitotic spindle microtubules are seen to be covered with stained PAP complexes, that are filling the clear zone normally present around them.

The cytoplasm contains evenly distributed PAP complexes. The mitochondria, the lumen of the endoplasmic reticulum, Golgi cisternae and vacuoles, and the bundles of microfilaments are completely unstained. The nuclear periphery, with the exception of the pores, shows pronounced staining. The nuclear content is negative. These preliminary observations are, however, subject to further investigation.

In conclusion, we have shown that it is possible to stain microtubules and most probably microtubular proteins in other configurations, with the PAP method using antibodies against purified rat brain tubulin. This method does not stain other fibrillar proteins such as actin or collagen.

This method is applicable at the light microscopic and ultrastructural level and provides a way of exploring the distribution of microtubules, tubulin and associated proteins and their interaction with other cellular components.

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Barbiturate reversal of amino acid antagonism produced by convulsant agents

THE hypnotic and anticonvulsant properties of pentobarbitone and related barbiturates may be closely associated with the actions of γ -aminobutyric acid (GABA)¹⁻³, an inhibitory transmitter at both pre- and postsynaptic receptors in the mammalian central nervous system⁴. Pentobarbitone prolongs pre-⁵ and postsynaptic³ inhibition as well as hyperpolarising central neurones^{2,3}. Although it has been suggested that pentobarbitone prolongs synaptic inhibition by delaying the removal of GABA from the synaptic cleft^{3,6} a direct action of pentobarbitone on GABA receptors cannot be excluded². We have explored this possibility both in the rat isolated sympathetic superior cervical ganglia preparation, which possesses GABA receptors analogous to those in the mammalian brain⁷, and *in vivo* on single neurones in the rat brainstem. In these experiments pentobarbitone, in amounts which produced no apparent GABA-mimetic effects, reversed the actions of bicuculline methochloride (BMC), a selective GABA antagonist.

Ganglia were isolated from Wistar rats under urethane anaesthesia and desheathed before superfusion with Krebs' solution (containing 2.6 μ M hyoscine) as described previously⁸. Surface potentials were monitored continuously through Ag⁺/AgCl electrodes in contact with the ganglion body and postganglionic trunk and connected across a Servoscribe 1-s potentiometric recorder.

The addition of GABA, at concentrations greater than 1 μ M, to the solution superfusing the ganglia produced a dose-dependent depolarisation. Pentobarbitone also depolarised ganglia but only at much higher concentrations. The threshold concentration was in excess of 80 μ M. Responses to pentobarbitone were less susceptible to the effects of BMC than were responses to equally effective doses of GABA.

Application of pentobarbitone during superfusion with BMC reversed the action of this GABA antagonist. An example of this reversal is shown in Fig. 1. Responses to GABA (30 μ M) were reduced approximately 50% in the presence of BMC (14 μ M). Addition of pentobarbitone in increasing concentrations reversed the effect of BMC. At this concentration of BMC the maximum reversal occurred generally at 80 μ M pentobarbitone. The effect of pentobarbitone was rapid in the onset, as well as offset; within 20 min following its removal responses to GABA were again reduced to the original antagonised level.

Pentobarbitone (80 μ M) applied in the absence of BMC (Fig. 1) generally did not produce responses to GABA administered at concentrations up to 30 μ M. But responses to larger concentrations of GABA were always markedly reduced. In spite of this, responses obtained to these concentrations of GABA, in the presence of both pentobarbitone and BMC, were always greater than in the presence of either substance alone.

The antagonism of responses to GABA produced by other substances, picrotoxin, tetramethylenedisulphotetramine and isopropyl bicyclopophosphate was also reversed by pentobarbitone whereas the antagonism of depolarising responses to carbachol (33 μ M), produced by hexamethonium (75 μ M), was not. In addition the non-selective depression of responses to GABA produced by strychnine⁷ (73 μ M) was reversed by pentobarbitone but the similar depression of responses to carbachol, produced by the same concentration of strychnine, was not reversed.

Other barbiturates also reversed the effects of the BMC. However, of those so far tested, only quinalbarbitone was found to be more potent (2-3 times) than pentobarbitone. The isomer of pentobarbitone, amylobarbitone, and the sulphur derivative thiopentone had similar activity to pentobarbitone whereas phenobarbitone was about 5% the potency.

This investigation was extended to *in vivo* experiments

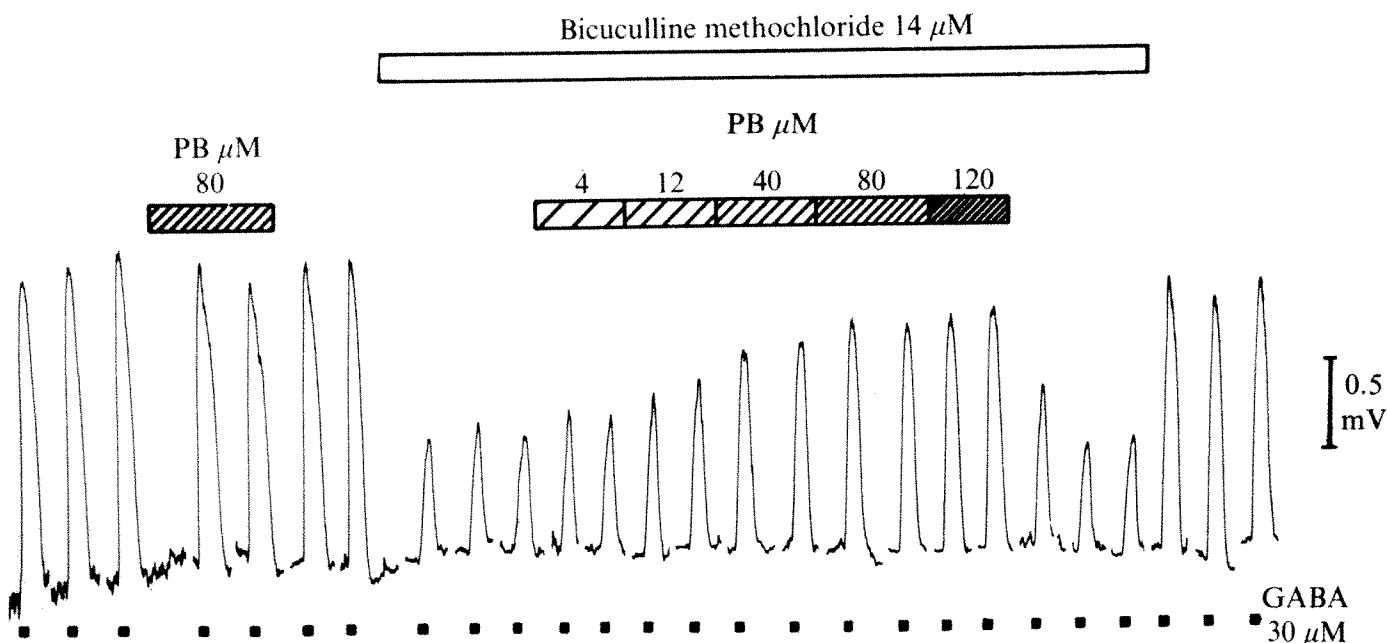


Fig. 1 Reversal by pentobarbitone of the antagonism of depolarising responses to GABA produced by bicuculline methochloride in an isolated superior cervical ganglion of the rat. The ganglion was superfused with Krebs' solution at 1 ml min⁻¹ and the surface potential measured as described previously^{7,8}. GABA (30 μ M) was applied for 1-min periods, indicated by the solid bars below the record, at intervals of 13 min. The recorder paper drive was switched off in between responses for periods of approximately 8 min (gaps in record). Pentobarbitone (PB, 4-120 μ M) and bicuculline methochloride (14 μ M) were present in the superfusing solution during the periods shown. Note that (1) pentobarbitone (80 μ M) applied in the absence of bicuculline methochloride neither depolarised the ganglion nor markedly altered responses to GABA, and that (2) increasing concentrations of pentobarbitone reversed the depression of responses to GABA produced by bicuculline methochloride.

performed on six adult albino rats (200–250 g) anaesthetised with intraperitoneal urethane (1.4 g kg^{-1}) and prepared for brainstem recording by the method of Bradley and Dray⁹.

Multibarrelled glass micropipettes were used to record extracellular action potentials from single spontaneously active neurones in the medulla and to administer substances close to them by electrophoresis. Micropipettes were filled immediately before use by a glass-fibre method or by centrifugation. The recording barrel contained 3 M sodium chloride. Another barrel containing 1 M sodium chloride was used to balance the net current at the electrode tip to 0 or to test for electrophoretic current effects. Other barrels contained freshly prepared solutions of GABA,

kg^{-1} , four cells). Pentobarbitone also reversed the non-specific antagonism of glycine by BMC on every occasion. The effects of pentobarbitone were never accompanied by significant changes in background firing and the antagonism of GABA by BMC was rapidly restored after the end of the pentobarbitone administration (Fig. 2). This antagonism of GABA could also be restored during a pentobarbitone administration by increasing the ejecting current of BMC.

Further tests with pentobarbitone alone, administered to the same neurones (six cells) on which a reversal of BMC antagonism had already been demonstrated, did not reveal any significant potentiation of submaximal responses to GABA or glycine. In fact, on occasions the responses to

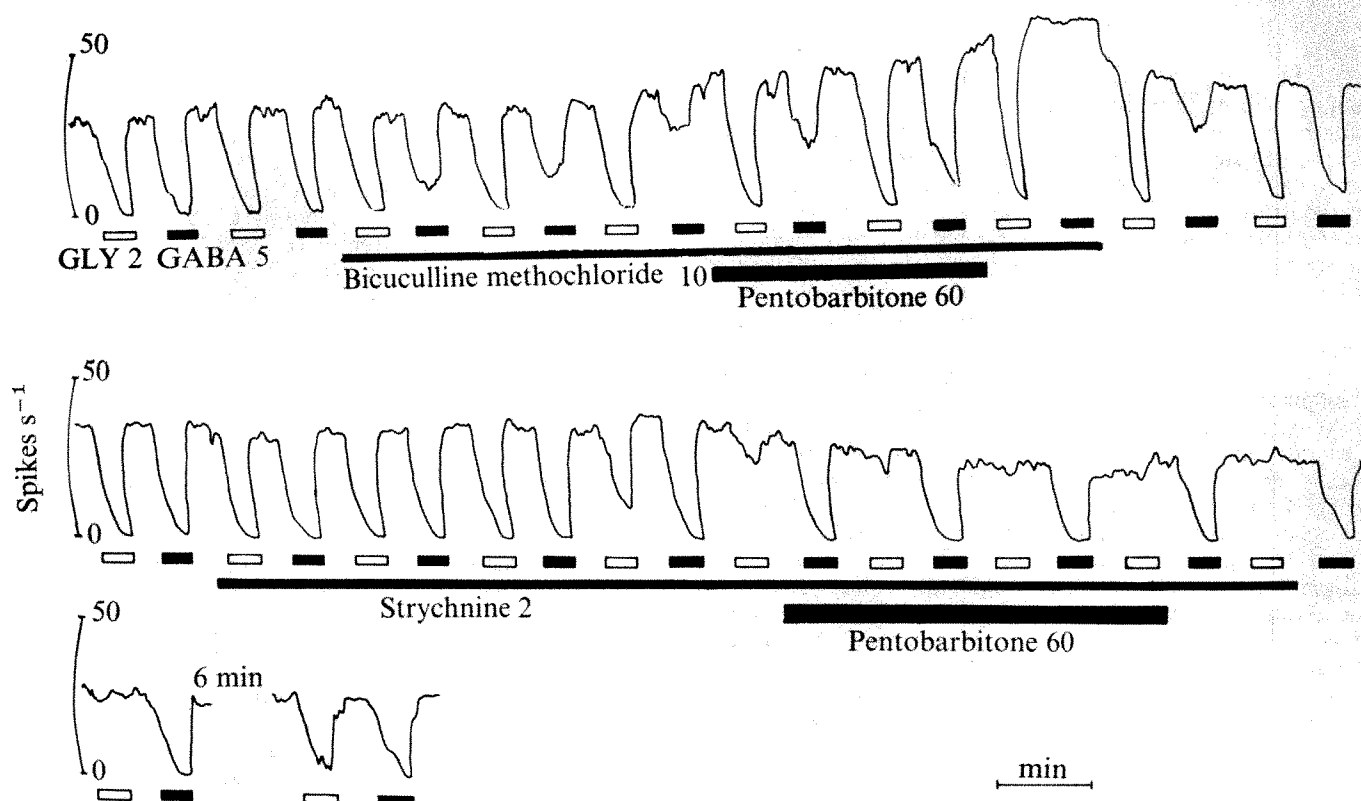


Fig. 2 Continuous ratemeter records of a spontaneously active neurone in the medulla showing the reversal by pentobarbitone of a selective antagonism of GABA by bicuculline methochloride. The GABA block by BMC was restored after the termination of the pentobarbitone administration. Strychnine selectively abolished the response to glycine on the same cell but this effect was not reversed by pentobarbitone. All expelling currents are indicated in nA.

0.2 M (pH 3.5); glycine, 0.2 M (pH 3.5); bicuculline methochloride, 5 mM (pH 3.5); strychnine, 5 mM (pH 3.5); pentobarbitone sodium, 20 mM (pH 9.5). Action potentials were recorded and displayed using conventional techniques.

Reproducible responses to GABA and glycine were obtained during consecutive applications of these compounds during a fixed time sequence. A continuous administration of BMC (10–20 nA) reduced the responses to GABA in all 19 neurones tested an example of which is shown in Fig. 2. In four of these cells responses to glycine were also partially reduced.

Pentobarbitone (40–80 nA, mean 56.6 nA; 1–6 min, mean 3.7 min) ejected from an adjacent barrel of the same micropipette during the continued administration of BMC partially or completely reversed the GABA antagonistic effect of BMC (Fig. 2). The reversal occurred in all neurones tested (26 tests on 19 neurones) and was also seen following intravenous administration of thiopentone (1 mg

GABA and glycine were reduced by the same current of pentobarbitone previously shown to reverse the antagonism produced by BMC. Larger currents ($>100 \text{ nA}$) of pentobarbitone produced marked depression of spontaneous firing.

Strychnine (2–5 nA) reversibly reduced the effects of glycine on 13 neurones and with larger ejecting currents (10–15 nA) additional reduction of the responses to GABA was observed (two cells). All tests with strychnine were performed on neurones which had also been tested before with BMC. The additional administration of pentobarbitone (30–70 nA; mean 64 nA; 2–13 min, mean 5.8 min) during a partial (50%) or complete suppression of glycine by strychnine, reversed strychnine antagonism in only five neurones. In most neurones strychnine antagonism of glycine was not affected although the amounts of pentobarbitone were in excess of those which had reversed BMC antagonism of GABA on the same cells. On the two cells where a non-

selective strychnine antagonism of GABA was observed, pentobarbitone readily and reversibly restored the GABA responses.

At present it is difficult to understand the mechanism by which barbiturates reverse the effect of BMC or other GABA antagonists. It might be argued that the inhibition of GABA uptake produced by pentobarbitone *in vitro*⁶ could account for the reversal. Uptake inhibition would increase the concentration of GABA in the vicinity of the receptors which might then overcome the competitive antagonism produced by BMC. But, uptake inhibition would also be expected to potentiate the responses to GABA even in the absence of BMC (compare the potentiating effect of nipecotic acid—ref. 10). However, no potentiation of responses to GABA was observed when pentobarbitone was administered in amounts which were subsequently shown to reverse the effect of BMC. In addition the presence of 3 mM nipecotic acid, which reduced ganglionic uptake of ³H-GABA (0.1 μ M) by more than 90%, did not affect the ability of pentobarbitone to reverse the action of BMC. An alternative explanation might be that GABA and BMC bind to the cell surface at different sites. If BMC binds at a site adjacent to the GABA site such that, when present, it prevents access of GABA to the receptor not because of preferential binding to the receptor but by either occluding it, or by distorting it. If pentobarbitone has an affinity for the antagonist binding site it could displace the BMC without itself affecting the receptor. GABA could then regain access to the receptor.

The possibility of a difference in agonist and antagonist binding sites is not without precedent. Snyder and Young¹¹⁻¹³ have suggested that glycine and strychnine bind to two separate but mutually interacting sites on synaptic membrane preparations of spinal cord and brainstem. They suggest that the antagonist binding site may, in fact, be associated with the ionic conductance mechanism for chloride whereas the agonist, glycine, interacts with the glycine recognition site. Snyder and his group¹⁴ have also demonstrated GABA receptor binding in similar preparations. However, as yet there is no evidence of separate binding sites for BMC and GABA. Interestingly, in this same study it was shown that pentobarbitone does not affect GABA binding. It remains to be seen whether pentobarbitone affects the binding of BMC in the same preparation.

The barbiturates are particularly useful in the treatment of convulsant poisoning^{15,16}. The present observations suggest that this action may be related to an effect at sites associated with inhibitory amino acid receptors.

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Digynic triploidy after superovulation in mice

SUPOVULATION through the administration of exogenous gonadotropins¹ has been widely used in mice for experimental purposes, and cytogenetic evidence has shown^{2,4} that the technique does not affect adversely the chromosome constitution of either released ova or zygotes. We have noticed, however, that the incidence of triploidy increased considerably among zygotes obtained through superovulation followed by natural mating in adult A-strain mice⁵. We have therefore investigated the origin of the extra genome in such embryos.

Six- to 8-week-old A/He females received 10 IU of pregnant mare serum (PMS, Schering) at 16.00 h and 10 IU of human chorionic gonadotropin (HCG, Schering) 44 h later. They were allowed to mate with CBA-T₆T₆ males after the second injection. Spontaneously ovulating and mated females served as controls. Eggs at the pronuclear stage were flushed from the oviduct early in the afternoon of the day when a copulation plug was found. Postimplantation embryos were dissected at 7.5 d of gestation from superovulated females, and at 6.5 or 7.5 d from control females. By changing the interval between HCG injection and death, we recovered preimplantation embryos from the eight-cell to the late blastocyst stage.

Totals of 430 and 1,423 viable embryos were obtained from 20 superovulated and 129 control females. Chromosomes were analysed in 421 and 1,406 cases, respectively (Table 1). Failure in nine and 17 embryos was due either to accidental loss of specimens or to the absence of metaphase cells. The frequency of diploid embryos was significantly lower in the superovulated than in the control group, the difference being ascribed to the fivefold increase in the incidence of digynic triploidy (Fig. 1). The frequency distribution of the two possible sex chromosome complements in digynic triploidy was 22XXX:25XXY:5? in the control and 30XXX:45XXY:6? in the superovulated group. The ratio in diandric triploidy was 4XXX:6XXY:1XYY in the control and 1XXY:1XYY in the experimental group, implying apparently reduced viability of XYY zygotes, as suggested in man⁶. Except for this specific class, digynic and diandric embryos, being chromosomally balanced, seemed to be of equal viability. It is, however, possible that more diandric eggs were lost early in development than digynic eggs, if dispermy occurred primarily in deteriorating oocytes. The incidence of triploidy (4.5%) in the control group was nearly comparable with that obtained in 'silver' mice. The high incidence was not ascribed to the interstrain cross¹⁰, for the situation was not appreciably different when strain A males were used. (N.T., unpublished results.)

The increase in digyny after superovulation would be due to suppression of either the first or the second polar body unless exogenous gonadotropins preferentially stimulated tetraploid oocytes. The nature of the polar bodies, and the number and relative size of the pronuclei¹¹, could reveal a disturbance at maturation division, an error at fertilisation or both. Only a small proportion of activated eggs had the first polar body in our preparation, which agrees with previous findings¹¹. Moreover, about one-tenth of the zygotes with a large (male) and a small (female) pronucleus possessed neither the first nor the second polar body. Inasmuch as these usually had excessively flattened cytoplasm, a loss

of the second polar body during preparation probably explains most of them. The two pronuclei were similar in size in a proportion of eggs from the control and the experimental group. Although variability in pronuclear size might account for most of such eggs, diploidy of the female pronucleus could not be ruled out.

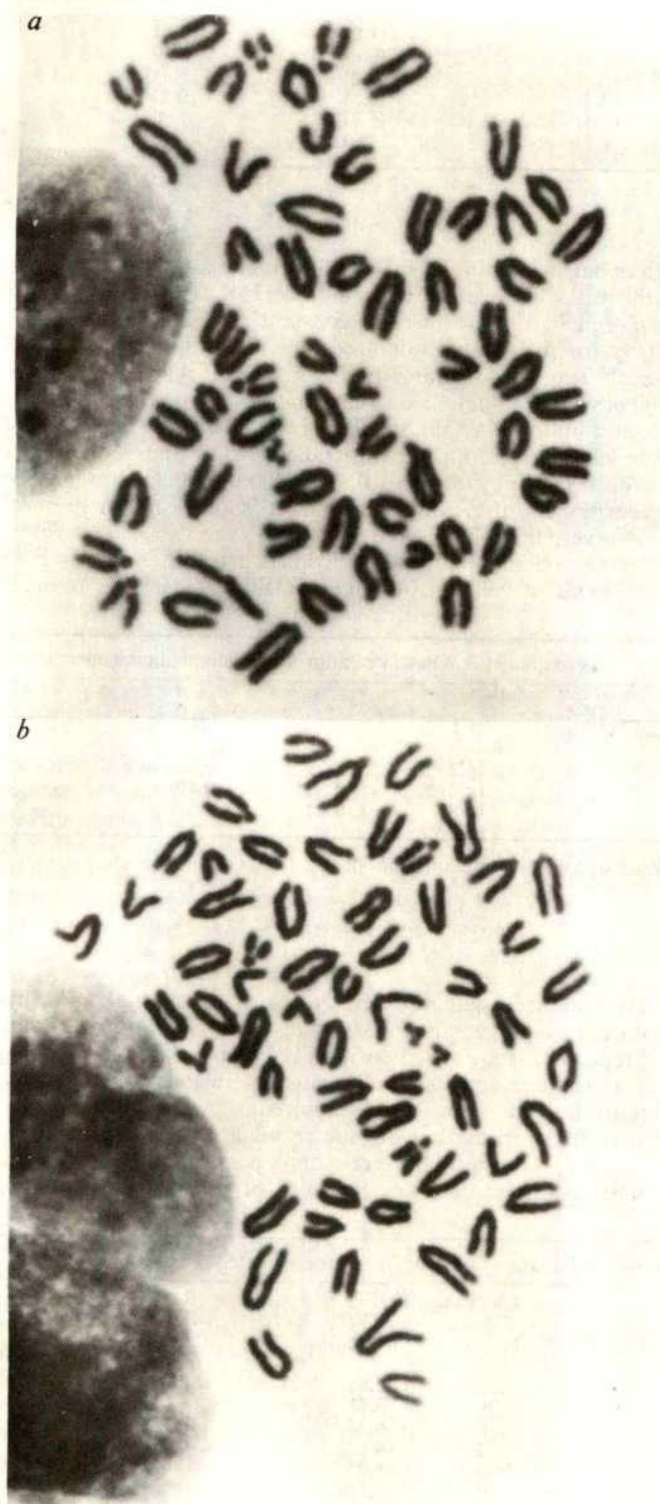


Fig. 1 Triploid metaphases from 7.5-d embryos dissected from A/He females mated with CBA-T₆T₆ males. *a*, Digynic triploidy with a single T₆ marker (arrow) and XXX sex chromosome constitution. *b*, Diandric triploidy with two T₆ markers (arrows) and XYY sex chromosome complex. Dissected embryos were incubated in Eagle's medium supplemented with foetal calf serum (20%) and Colcemid (0.1 µg ml⁻¹) for 2 h. Chromosome preparations were made according to the method of Wroblewska and Dyban⁶ with modifications⁷.

A total of 354 of 1,264 superovulated eggs had three pronuclei. Digyny was presumed to occur in 333 and 254 eggs on the basis of the absence of the second polar body and the difference in pronuclear size, respectively. There were 21 diandric (dispermic) eggs estimated on the basis of the retained second polar body, and 49 on the basis of pronuclear size (Fig. 2). Divergence between the two estimates in both classes of eggs may diminish considerably if loss of the second polar body and the variability in pronuclear size are taken into account. The interpretation of our data may be that (1) most triploid embryos identified in later stages derive from zygotes with three pronuclei; (2) digyny outnumbers diandry, and (3) disturbance of the second maturation division is the major cause of digyny. All of the zygotes with three pronuclei found in the control mating were compatible with suppression of the second polar body. Eight zygotes with four pronuclei presumably represented dispermic eggs whose second polar body was suppressed.

Fig. 2 Presumptive triploid zygotes at the pronuclear stage. Two small (female) pronuclei with a single nucleolus in (*a*) suggest digyny due to suppression of the second polar body, and two large (male) pronuclei with several nucleoli in (*b*) suggest dispermic. Possibly the second polar body was lost during preparation.

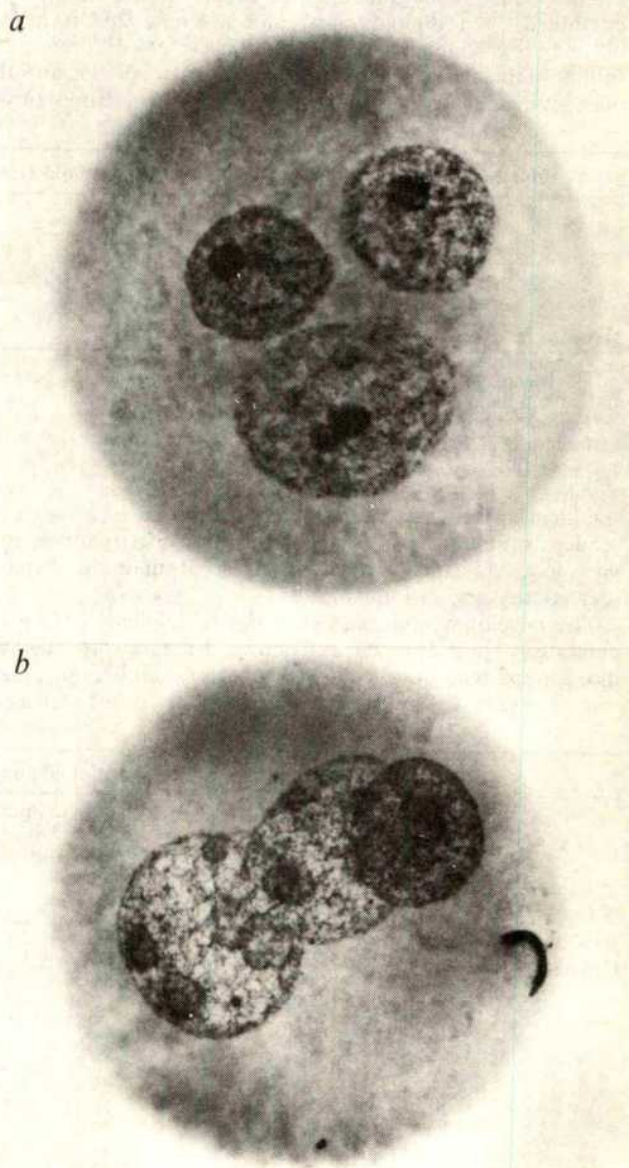


Table 1 Chromosome findings in 6.5–7.5-d embryos from control and superovulated females mated with CBA-T₆T₆ males

	Control	Superovulated	χ^2_c
No. of litters	129	20	
No. of embryos recovered (mean)	1,423 (10.9)	430 (21.5)	
No. of embryos karyotyped (%)	1,406 (98.8)	421 (97.9)	
2n (%)	1,298 (92.3)	320 (76.0)	83.29†
2n-1 (%)	12* (0.9)	5 (1.2)	0.12
2n+1 (%)	20 (1.4)	4 (1.0)	0.24
Mosaics (%)	9 (0.6)	9 (0.6)	1.50
3n, T ₆ /+/+ (%)	52 (3.7)	81 (19.2)	113.98†
3n, T ₆ /T ₆ /+ (%)	11 (0.8)	2 (0.5)	0.11
4n, T ₆ /T ₆ /+/+ (%)	4 (0.4)	3 (0.7)	0.66
Total heteroploids (%)	108 (7.7)	101 (24.0)	83.29†

*Including one 2n-2.

† $P \leq 0.005$.

The proportion of informative embryos was low at the morula–blastocyst stage because of technical difficulties. We obtained 1,022 informative cases including 219 triploids. As diagnosis of aneuploidy was difficult at this stage, embryos with 38–41 chromosomes were classified as diploid. The incidence of diploid, triploid and tetraploid embryos at pronuclear, morula–blastocyst, and postimplantation stages are compared in Table 2. To make data comparable, genuine diploids, aneuploids and mosaics in the diploid range were combined as a diploid class, and a single 2n/3n mosaic blastocyst was included in a triploid class. No difference was found in the three classes of embryos between the morula–blastocyst and the postimplantation stage, but triploidy was

numbers according to Edwards' formula¹². As Table 3 shows, the ratio between cleavage numbers of triploids and those of diploid sibs was nearly constant, ranging from 0.88 to 0.92 for the stages examined. This may imply that the cell-cycle time was about 10% longer in triploids than in diploids. Extrapolation of this relationship predicts that the cell number of triploid embryos would become half that of diploid sibs at 10th cleavage (about 5.5 d) and a quarter at 20th cleavage (about 10.5 d), though delay might be accelerated after implantation. It should be mentioned, however, that there were substantial variations in developmental potential among individual triploid embryos^{2,13–15}.

Foetal development, investigated extensively in mice

Table 2 Frequency of diploid, triploid and tetraploid embryos from superovulated females in relation to developmental stages

	Pronuclear	Morula–blastocyte	Postimplantation	I	χ^2_c II	III
2n	870	799*	335*	16.20†	12.29†	0.27
3n	354	220†	83	14.87†	12.66†	0.49
4n	8	3	3	0.83	0.04	0.31

I, Between pronuclear and morula–blastocyst stages; II, between pronuclear and postimplantation stages; III, between morula–blastocyst and postimplantation stages.

*Embryos within the diploid range.

†Including a 2n/3n mosaic.

‡ $P \leq 0.005$.

significantly more frequent at the pronuclear stage. Consequently we conclude that a proportion of triploid zygotes was lost in early cleavage stages. Remaining triploids seemed to survive implantation.

The developmental characteristics of triploids before implantation were assessed as follows. Litters were classified into groups according to the average cell numbers of diploid sibs. Average cell numbers were converted into cleavage

after induced ovulation, has revealed substantial mortality during cleavage, about the time of implantation, at mid-pregnancy and at parturition^{16–18}. The extent to which chromosome imbalances is causally related to embryonic mortality has not been explored yet. Our observation strongly suggests that digynic triploids constitute an appreciable proportion of pre- and postimplantation losses. Possibly the same holds for mice of other strains, even if

Table 3 Cell and cleavage numbers in diploid and triploid preimplantation embryos from superovulated females

Cell numbers \pm s.e.m.	(No. of embryos)	Cleavage numbers		Ratio
I	II	III	IV	IV/III
diploid	triploid	diploid	triploid	
9.23 \pm 0.32 (17)	7 (1)	3.21	2.81	0.88
16.27 \pm 0.64 (49)	13.10 \pm 1.37 (10)	4.06	3.71	0.91
27.52 \pm 1.40 (29)	20.56 \pm 2.17 (9)	4.78	4.36	0.91
32.46 \pm 1.55 (35)	24.56 \pm 1.73 (16)	5.02	4.62	0.92
44.88 \pm 1.24 (82)	32.88 \pm 1.05 (40)	5.50	5.04	0.92
55.23 \pm 1.63 (43)	36.38 \pm 4.50 (8)	5.79	5.19	0.90
63.54 \pm 1.52 (84)	43.87 \pm 2.98 (37)	5.99	5.46	0.91
76.19 \pm 1.98 (72)	53.83 \pm 3.55 (12)	6.25	5.75	0.92
83.82 \pm 1.72 (86)	56.71 \pm 3.60 (14)	6.39	5.83	0.91
97.05 \pm 2.27 (37)	63.42 \pm 2.78 (26)	6.60	5.99	0.91
109.67 \pm 5.22 (21)	71.33 \pm 11.85 (3)	6.78	6.16	0.90
131.37 \pm 3.41 (63)	80.27 \pm 3.51 (22)	7.04	6.33	0.90
147.17 \pm 2.76 (97)	95.26 \pm 4.48 (27)	7.20	6.57	0.91
167.32 \pm 3.46 (37)	100.50 \pm 6.77 (8)	7.39	6.65	0.90
186.15 \pm 12.89 (13)	102.39 \pm 9.94 (7)	7.54	6.68	0.89

III = $\log I/\log 2$; IV = $\log II/\log 2$.

the frequency of triploids is less pronounced than in the strain we used. This assumption is supported by two lines of evidence. These are (1) high incidence of chromosome abnormalities, including triploidy, in rabbit blastocysts obtained through superovulation by PMS¹⁹, and (2) the increased frequency of triploidy and other types of chromosome anomalies observed in human spontaneous abortuses after ovulation-inducing therapy by HMG and HCG, clomiphene citrate and HCG, or HCG alone²⁰.

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Selective inhibition of neuronal GABA uptake by *cis*-1,3-aminocyclohexane carboxylic acid

It is now generally accepted that GABA is an important inhibitory synaptic transmitter in the vertebrate central nervous system. However, there remain many problems in elucidating the precise roles of GABA in the brain. One important problem is the existence of a glial pool of GABA which makes it difficult to determine the origin of GABA released during experiments designed to demonstrate the release of GABA after nervous stimulation. It was thought at one time that this problem might be overcome by utilising the uptake processes for GABA to label the neuronal GABA pools, with for example, ³H-GABA. However, it is now clear that in many areas of the brain, GABA is also taken up by glial cells and in some areas, this glial uptake may actually predominate. The transport processes for GABA in neurones and glia have remarkably similar properties but do appear to have slightly different structural requirements.

In the present study, we have found that a conformationally restricted structural analogue of GABA, *cis*-1,3-aminocyclohexane carboxylic acid (ACHC) (Fig. 1), which has previously been reported to competitively inhibit GABA transport in slices of cerebral cortex¹, selectively inhibits neuronal transport of GABA and has little or no effect on transport of GABA into glial cells. This selective inhibition was revealed by studying the uptake of ³H-GABA by isolated slices of rat cerebral cortex, frog retinae, rat retinae and rat sympathetic ganglia. Autoradiographic studies with ³H-GABA have shown a predominantly neuronal uptake in

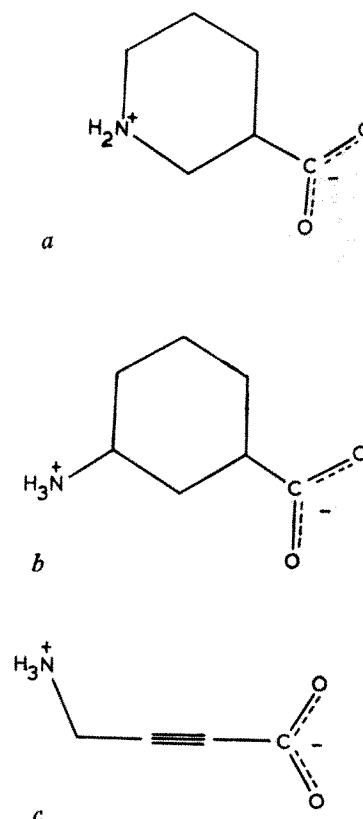


Fig. 1 Chemical line diagrams for nipecotic acid ((±)-piperidine-3-carboxylic acid) (a), ((±)-*cis*-3-aminocyclohexane carboxylic acid) (b), 4-aminotetrotic acid (4-aminobut-2-ynoic acid) (c).

cortical slices¹ and frog retinae³ and a glial uptake in rat retinae⁴ and ganglia⁵.

The effects of ACHC on ³H-GABA uptake by these tissues are summarised in Table 1. It can be seen that the uptake of ³H-GABA (0.1 μM) by cortical slices and frog retinae was 50% inhibited (IC₅₀) by ACHC at a concentration of 62 μM and 960 μM respectively but uptake into rat retinae and ganglia was unaffected by ACHC at a concentration of 1 mM. Since this concentration of ACHC was approximately 100 times the apparent K_m of the high-affinity GABA transport processes, these results indicate that ACHC has no significant inhibitory effect on GABA transport in glia of the rat retina and ganglion.

To study further the selectivity of ACHC on neuronal and glial systems, its ability to stimulate ³H-GABA release was investigated. In these experiments the tissue was pre-loaded with ³H-GABA and then superfused in a small chamber with medium containing aminooxyacetic acid (0.1 mM) to prevent catabolism of the accumulated ³H-GABA. During the superfusion the tissue was exposed for 4- or 6-min periods to medium containing ACHC or non-radioactive GABA. ACHC caused an increase in the efflux of ³H-GABA from superfused cortical slices and frog retinae (Fig. 2). This effect of ACHC was concentration dependent. Thus, in both tissues ACHC (10 μM) produced an approximately twofold increase in the efflux rate coefficient and this was increased further to about fivefold at a concentration of ACHC of 100 μM (cortex 5.2 ± 0.21, frog retina 4.5 ± 0.16, mean ± s.e.m. of six experiments). At 1 mM ACHC, much larger effects were seen, the rate coefficients increasing more than 14-fold (cortex 14.9 ± 1.21, retina 14.1 ± 2.01, mean ± s.e.m. 3-6 experiments). In contrast to the above tissues in which neuronal uptake of GABA predominates, the efflux of ³H-GABA from glial cells of the rat retina and ganglion was virtually unaffected by ACHC at concentrations up to 1 mM although GABA

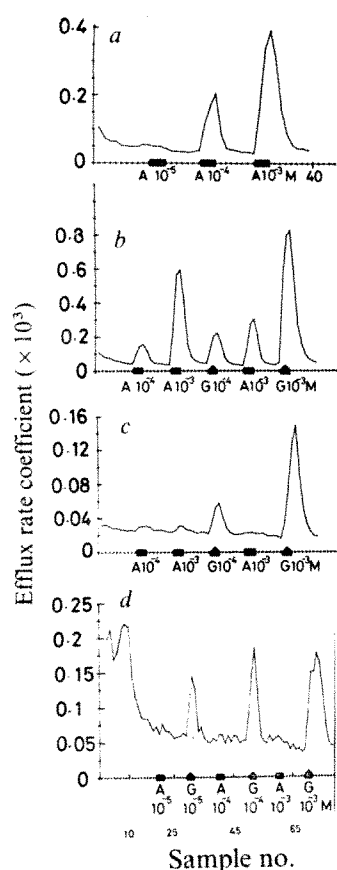


Fig. 2 Effect of GABA (G) and ACHC (A) on the rate of ^3H -GABA efflux from cerebral cortex slices (a), frog retina (b), rat retina (c), and rat sympathetic ganglion (d). Tissues were given a preliminary incubation for 10 min in 5 ml Krebs' bicarbonate solution at 25 °C. ^3H -GABA was then added (final concentration 1 μM) and the incubation continued for 30 min (ganglia 2 h). The tissue was then transferred to a superfusion chamber (volume 1.0 ml). Four slices of cortex (0.25 mm thick) or two retinæ were placed in each chamber and superfused at 25 °C with oxygenated medium at a rate of 1 ml min⁻¹. Fractions (2-ml) were collected and the radioactivity determined by liquid scintillation counting. The efflux of ^3H -GABA from sympathetic ganglia was measured as described previously¹⁴: ganglia were superfused with medium at 0.5 ml min⁻¹, and the radioactivity in 2-min samples determined. The results are expressed as the fractional rate coefficient defined as the amount of radioactivity collected during a 2-min period divided by the arithmetic mean of the total radioactivity present in the tissue at the beginning and end of the same collection period. All media contained the GABA transaminase inhibitor, amino-oxyacetic acid, 100 μM (ganglia 10 μM), as previous experiments have shown that in the presence of this inhibitor the radioactivity accumulated and released by these tissues corresponds to > 95% ^3H -GABA (refs 14 and 20-22).

(1 mM) itself produced large increases in the efflux of ^3H -GABA from all four tissues (Fig. 2).

The stimulated efflux of ^3H -GABA produced by ACHC and by GABA itself was dependent on the presence of Na⁺ ions in the medium. In the absence of Na⁺, the spontaneous release from the tissue was increased sixfold but the addition of GABA (1 mM) or ACHC (1 mM) did not cause any additional release of ^3H -GABA (Fig. 3). These effects were not due to damage of the tissue because when the Na⁺ level was restored, a stimulated efflux of ^3H -GABA was obtained with both GABA and ACHC (Fig. 3). These results suggest that the increased efflux of ^3H -GABA produced by ACHC (and GABA) is due to inhibition of the GABA transport process which is presumably

'recapturing' a large proportion of the spontaneously released ^3H -GABA. The alternative possibility that the stimulated release of ^3H -GABA is due to a coupled exchange process⁶ with the ACHC or exogenous GABA is unlikely since in the absence of sodium ions GABA uptake is prevented and this would be expected to reduce the spontaneous GABA efflux rather than cause the marked increase that was found in the present experiments.

The above experiments indicate that (in the tissues studied) ACHC is a selective inhibitor of neuronal GABA transport and is capable of producing a net increase in the efflux of GABA from neuronal but not glial GABA pools. The properties of ACHC which confer this specificity are of interest. In aqueous solution ACHC is likely to exist as the zwitterionic form and from *pK* measurements it has been concluded that the molecule exists predominantly in the diequatorial conformation⁷. In this conformation the compound represents a partially restricted, partially folded analogue of GABA where the separation of zwitterionic centres (mean of the two N...O distances) is approximately 5.6 Å. The corresponding distance observed in the crystal structure of β -alanine is approximately 3.8 Å (ref. 8). Since β -alanine seems mainly to inhibit glial uptake of GABA⁹, the selectivity of inhibitors of GABA uptake is probably related to the separation of their zwitterionic

Table 1 Effect of ACHC and other analogues on GABA uptake

Analogue	IC ₅₀ (Concentration of compound that inhibits uptake of GABA 0.1 μM by 50%)			
	'Neuronal uptake'		'Glial uptake'	
	Cortical slices	Frog retina	Rat retina	Sympathetic ganglia
<i>cis</i> -1,3-aminocyclohexane carboxylic acid	62 μM	960 μM	No effect at 1 mM	No effect at 1 mM
4-Aminotetrollic acid	330 μM	Not tested	No effect at 1 mM	No effect at 1 mM (ref. 15)
Nipecotic acid	12 μM	1 mM	640 μM	200 μM
2,4-Diaminobutyric acid	66 μM (ref. 16)	Not tested	58 μM (ref. 17)	No effect at 1 mM (ref. 10)
β -alanine	21 mM (ref. 9)	Not tested	390 μM (ref. 18)	59 μM (ref. 14)

Slices (0.1 × 0.1 × 0.2 mm) of cerebral cortex (10 mg)¹⁹ or individual retinæ (10 mg)¹⁷ were preincubated with analogue for 10 min at 25 °C in 5 ml Krebs' bicarbonate medium. ^3H -GABA was then added to give a final concentration of 0.1 μM and the incubation continued for 10 min. Desheathed ganglia¹⁰ were incubated in Krebs' solution at 25 °C for 30 min with ^3H -GABA (0.1 μM) in the absence or presence of analogue. The tissue was then recovered, washed with fresh medium at 25 °C, and transferred to counting vials. The radioactivity was extracted by dissolving the tissue in Soluene-350 (Packard) and measured by liquid scintillation counting. The counts were corrected for efficiency by the channel ratio method. The inhibition of ^3H -GABA uptake produced by 3 or 4 concentrations of each analogue was measured and used to obtain the IC₅₀ value¹⁸. The value at each concentration of analogue was the mean of 3-6 results and the s.e.m.s were less than 10%.

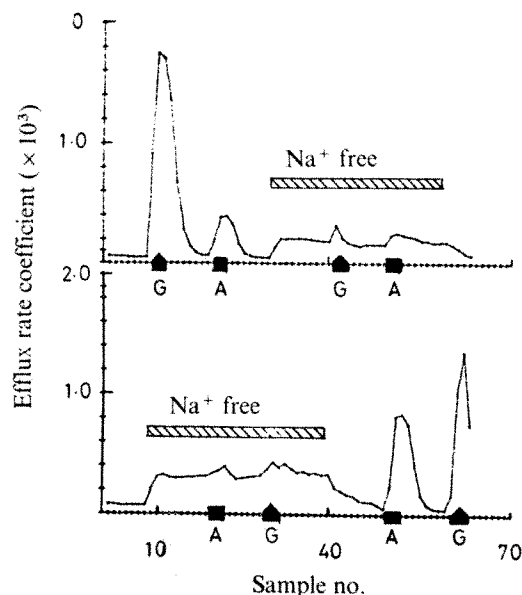


Fig. 3 Effect of Na^+ ions on the stimulated release of ^3H -GABA from cortical slices produced by ACHC (1 mM) and GABA (1 mM). Experimental details were as described in Fig. 2 except that a Tris-HCl buffered medium was used and where indicated the Na^+ in the medium was replaced with sucrose. The top panel shows that the stimulated release of ^3H -GABA produced by GABA (G) and ACHC (A) was abolished by exposure of the tissue to Na^+ free medium (indicated by the solid bar). This lack of response was not due to tissue damage because the stimulated release of ^3H -GABA was restored when the Na^+ -free medium was replaced with normal medium (bottom panel). Note also the increased spontaneous efflux of ^3H -GABA in the absence of Na^+ ions.

centres. The upper limit of this separation for interaction at glial uptake sites is apparently lower than at neuronal uptake sites. This is supported by the fact that 4-aminotetrolic acid (4ATA), where the zwitterionic centres have a minimum separation of 5.5 Å was also found to be a selective neuronal inhibitor of GABA uptake (Table 1). In contrast, nipecotic acid, which like ACHC, also adopts the chair conformation in both the solid state and aqueous solution but has a smaller separation of charged centres of approximately 4.5 Å, interacts with both neuronal and glial transport sites for GABA (Table 1).

2,4-Diaminobutyric acid (DABA) has previously been suggested as a selective inhibitor of neuronal GABA transport because it inhibits uptake by cortical slices but not by sensory⁹ and sympathetic ganglia¹⁰. However, DABA is almost equally potent in inhibiting GABA uptake in rat retina and cortical slices. This lack of selectivity is presumably due to the greater flexibility of this molecule.

In addition to its affinity for neuronal GABA transport sites, ACHC has been shown to have an inhibitory effect on the firing of central neurones. This effect is presumably mediated by GABA receptors since it is blocked by the GABA antagonist, bicuculline¹¹ (M. A. Simmonds and F. Andres-Trelles, personal communication). In contrast, we found that ACHC (3 mM) had no effect on ganglionic GABA receptors since it failed to produce the neuronal depolarising response characteristic of GABA itself¹² (Fig. 4). Nipecotic acid (3 mM) also had no effect on ganglionic neurones but depolarising responses were produced by relatively high concentrations of DABA (3 mM) and β -aminobutyric acid (BABA) (3 mM) (Fig. 4).

When the ganglionic glial cells were allowed to accumulate GABA by superfusion of the ganglion with 1 mM GABA for 1 h (ref. 14), it was found that the depolarisations produced by DABA and BABA were potentiated and

nipecotic acid, which was previously without effect, now produced a prolonged depolarisation. In contrast, ACHC still did not depolarise the ganglionic neurones (Fig. 4). The potentiation of the depolarising responses of DABA and BABA after loading of the glial cells with GABA is due to their affinity for the glial transport sites. These compounds inhibit the reuptake of GABA and so increase the net release of GABA from the glial cells as illustrated previously (Fig. 2). This indirect activation of ganglionic GABA receptors is even more strikingly demonstrated in the case of nipecotic acid which produced no depolarisation at all until after the glial cells were loaded with GABA but then produced a prolonged depolarisation of the ganglionic neurones (Fig. 4). Unlike nipecotic acid, ACHC did not depolarise ganglionic neurones after the glia were loaded with GABA. This failure to show an indirect effect on ganglionic GABA receptors is consistent with this compound's lack of affinity for glial uptake sites. However, since

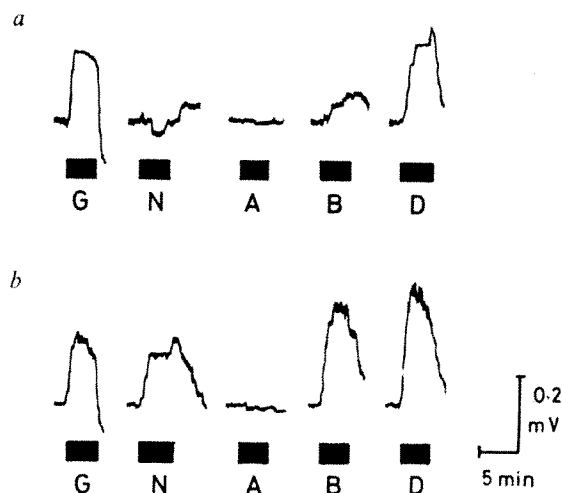


Fig. 4 Depolarising responses of an isolated superior cervical ganglion to GABA (G, 3 μM), ACHC (A, 3 mM), β -aminobutyric acid (B, 3 mM), 2,4-diaminobutyric acid (D, 3 mM) and nipecotic acid (N, 3 mM). The ganglion was obtained from a Wistar rat under urethane anaesthesia and desheathed before superfusion with Krebs' solution (1 ml min⁻¹) at 25 °C. Two non-polarisable Ag^+/AgCl electrodes were placed in contact with the postganglionic trunk and ganglion body and connected across a Servoscribe 1-s potentiometric recorder¹³. Responses correspond to an increase in the potential difference measured across these electrodes. Responses were obtained before (a) and after (b) superfusion of the ganglion with 1 mM GABA for 60 min followed by a further 60 min superfusion with Krebs' solution¹⁴. Each drug was in contact with the tissue for 4 min, indicated by the solid bars below the record, and was applied at intervals of 15–20 min (gaps between records). Aminooxyacetic acid (10 μM) was present in all solutions. Note that in b responses to nipecotic acid, β -aminobutyric acid and 2,4-diaminobutyric acid were larger than in a, whereas ACHC still failed to evoke a response.

ACHC does have affinity for neuronal GABA transport sites, this raises the interesting possibility that the inhibitory effects of this compound (and perhaps nipecotic acid) on the firing of central neurones is mediated indirectly by increasing the net efflux of endogenous GABA from neuronal pools. An alternative possibility which cannot be excluded at present is that the receptors for GABA on ganglia and on central neurones have different specificity requirements.

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Distribution of ions in a fluid-transporting epithelium determined by electron-probe X-ray microanalysis

STUDIES of fluid transport in epithelia have in general been limited to measurements of the composition of the transported fluid and the electrochemical gradients against which transport occurs¹⁻³. We have now used electron-probe X-ray microanalysis to measure the intracellular ionic concentrations. The data show that Na, K and Cl are not uniformly distributed within the cells; that the basal lamina is not entirely a passive membrane open to small ions, and that, in the particular epithelium studied, the stimulation of secretion greatly increases the intracellular Na concentration. In addition, the results do not support the standing gradient theory of fluid secretion.

Our technique (see legend to Fig. 1) is a refinement of that reported earlier⁴: tissue in known physiological condition is quench-frozen at -180°C , sectioned and transferred to the cold stage of an X-ray microanalyser. By the maintenance of low temperature, the water in the tissue is retained as ice and the distribution of soluble constituents is preserved—as has now been confirmed by experiments on several animal tissues (ref. 5, and our unpublished work). No chemical fixation or dehydration is used.

We studied the fluid-secreting upper portion of Malpighian tubules from the blood-sucking insect *Rhodnius prolixus*. Depending on the composition of the bathing medium, this epithelium secretes fluid rich in either Na or K or both⁶. Furthermore, the normally low rate of fluid secretion can be increased (up to 1,000 times) by treatment with diuretic hormone from the insect, or with 5-hydroxytryptamine (5-HT)^{7,8}.

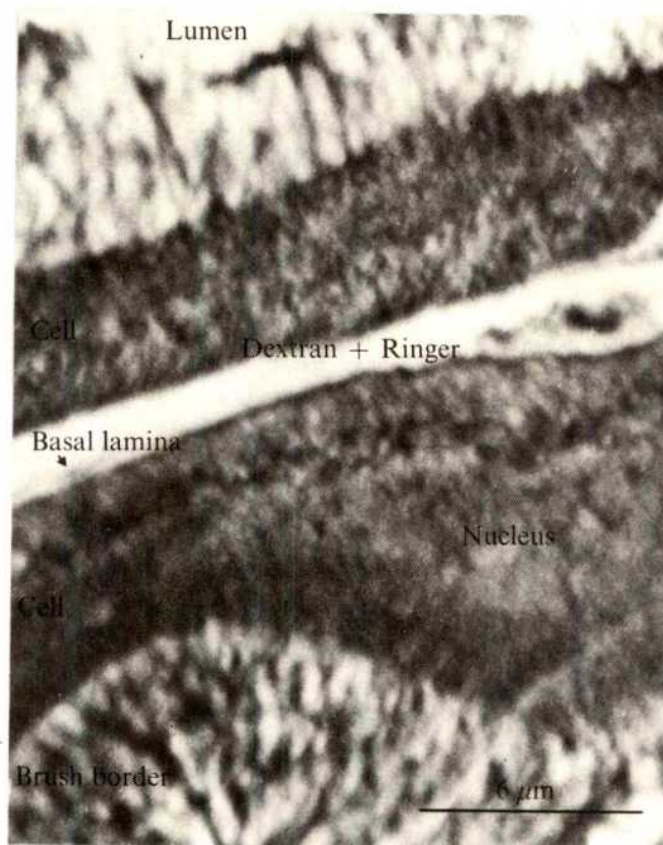


Fig. 1 Scanning transmission electron micrograph of a deep-frozen hydrated section of the upper secretory portion of a Malpighian tubule from *Rhodnius prolixus*, showing two parts of a tubule, side by side, with various tissue components as labelled. Because of the wide range of contrast in the image, the basal lamina is not well shown in this micrograph. Tubules from unfed, fifth-instar *Rhodnius* were dissected in saline containing (mM) K^+ 20, Na^+ 132, Cl^- 160, Ca^{2+} 2, Mg^{2+} 9, H_2PO_4^- 4, HCO_3^- 10, glucose 34, together with 10–20% (w/v) dextran (molecular weight about 237,000). The dextran was included to reduce ice-crystal damage during quenching and to improve sectioning at low temperature; physiological experiments showed that it had no significant effect on secretion rates. For stimulated tubules, about 10^{-5} M 5-HT was added. (Other tubules from the same insect were isolated in identical saline containing ^{22}Na or ^{36}Cl , so that the concentrations of these ions in the secreted fluid could be measured for comparison with the microprobe results.) Lengths of tubules were mounted in a drop of dextran-saline on either silver or copper pins from a modified Cryokit attachment for the LKB Ultratome III (B. L. G. and N. G. F. Cooper, in preparation) or on small chunks for a cryomicrotome (SLEE Instrument Co.)⁴. They were then quench frozen at -180°C by plunging into Freon-13 (monochlorotrifluoromethane) maintained as a solid-liquid slush with liquid nitrogen, and transferred to the microtome. Sections (1–2 μm thick) were cut with steel knives at -80°C in the SLEE microtome, or with glass knives at -120° to -140°C in the LKB Cryokit. The sections were picked up on aluminium alloy collars covered with aluminised nylon films and loaded on to the low-temperature stage of a JEOL JXA-50A X-ray microanalyser. Special transfer devices were used to maintain the sections below -150°C to protect them from dehydration. Throughout the observations the specimen stage was kept at -170°C ; in these conditions the loss of mass from areas under the beam is unlikely to be significant^{1,26}. After each set of microanalyses the state of hydration of the section was checked as follows: the specimen holder was removed from the cold stage and held at the end of the loading rod within the column under high vacuum for 1–2 min. During this time the section warmed up and dried rapidly. The holder was then returned to the cold stage and analysis was repeated. From the change in X-ray continuum after drying, without parallel changes in either the characteristic counts or the distribution of elements, the section illustrated here and the other sections used for the results in Figs 2 and 3 were estimated to have retained 90–80% of their original water contents during the first analysis.

Figure 1 is a scanning transmission electron micrograph of a 1- μ m thick, deep-frozen, hydrated section of a *Rhodnius* Malpighian tubule stimulated with 5-HT and prepared as described in the legend. To analyse the distribution of elements, a focused electron beam about 100 nm in diameter was localised in different areas at magnifications of 5,000–20,000. Characteristic X-ray signals from the K α radiations of Na and K were recorded from diffracting spectrometers while a complete X-ray energy spectrum was obtained simultaneously from a Kevex Si(Li) detector. The energy spectrum provided data on K, Cl and other elements and on the continuum or "white" radiation used as a measure of local mass in the specimen⁹. After correction for background and for contributions to the mass counts from the substrate film and the specimen holder, each measurement was converted into an elemental mass fraction by comparison with the corresponding counts for known concentrations in dextran-saline in the same section^{9,10}. These values are given as mmol per kg wet weight of the areas indicated in Figs 2 and 3, which summarise the results of several experiments with both unstimulated and 5-HT-stimulated tubules. The salient results are as follows.

The fluid in the lumen of unstimulated tubules was different from the fluid secreted at a high rate by stimulated tubules. The former was much richer in potassium, much

poorer in sodium, and poorer in chlorine. It also contained a high concentration of phosphorus, presumably as inorganic phosphate.

In the cytosol the distribution of ions was non-uniform, but with respect to the main cell cytoplasm (areas 5 and 6 in Fig. 2), the only significant consequence of stimulation was an increase of about 30 mM in intracellular Na.

In the region of the brush border, our first analysis (areas 9–11 in Fig. 2) did not distinguish between the microvilli and the adjacent extracellular spaces; these data do not show a systematic variation in Na, K or Cl with distance into the lumen in the unstimulated tubules, but in the stimulated tubules the levels of all three ions increase towards the lumen. With more careful localisation of the probe (Fig. 3) we found significant differences between the microvilli and the extracellular spaces, and gradients along the length of both. In stimulated tubules, the increase of Na and K towards the lumen was particularly marked, so that both elements seemed to be significantly more concentrated towards the luminal end of the extracellular space than in the main lumen.

The basal lamina contained a higher concentration of K and lower concentrations of Na and Cl than the dextran-saline (Fig. 2, area 2). Phosphorus concentrations in the lamina were higher than elsewhere.

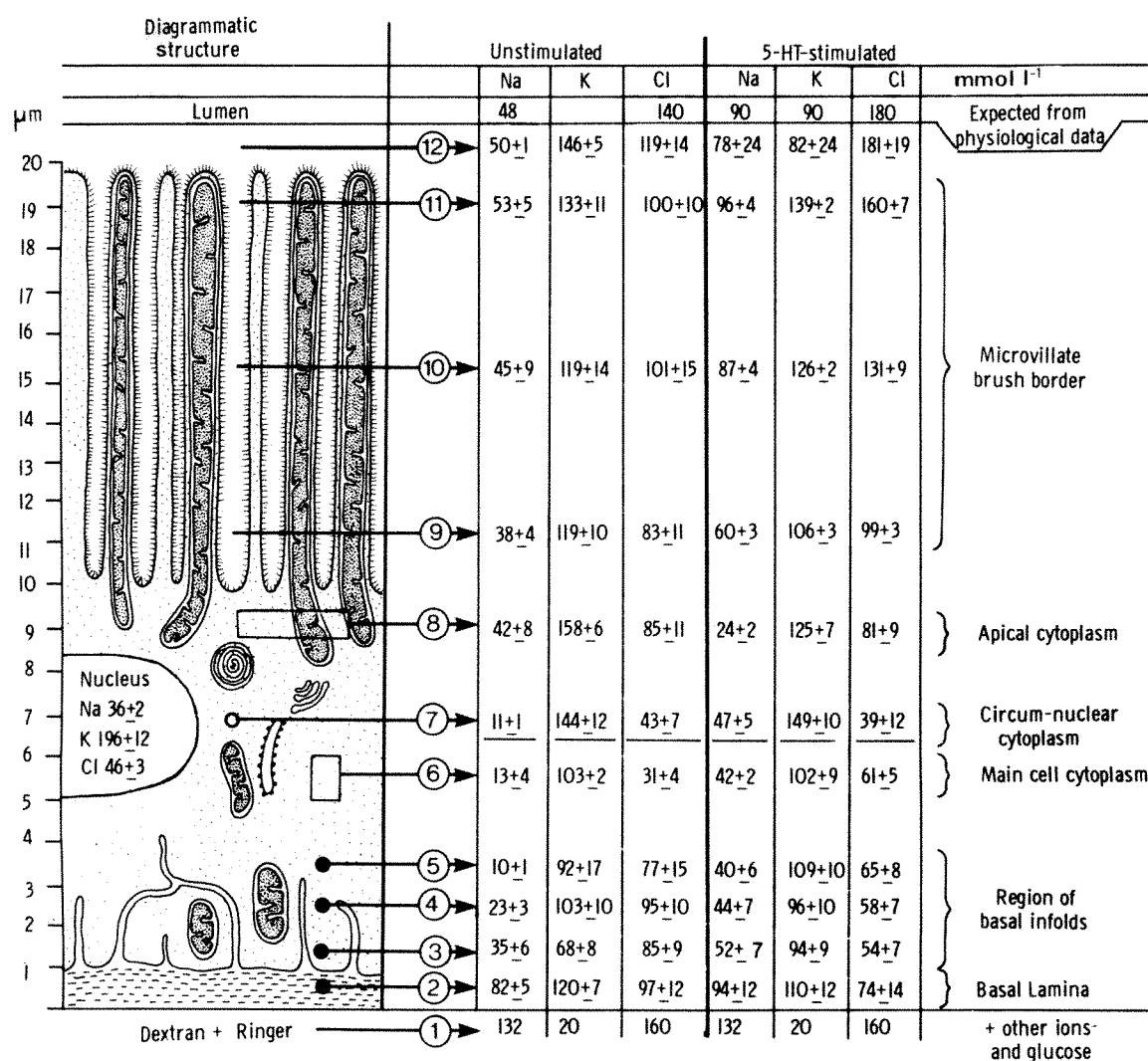


Fig. 2 Summary of microanalytical results obtained from stimulated and unstimulated Malpighian tubules of *Rhodnius*. The diagrammatic representation of the structure was prepared from conventional electron micrographs, and shows positions (but not actual sizes) of areas scanned by the probe. Measured concentrations of Na, K and Cl are expressed in mM (\pm 1 s.e.) per kg wet weight. The contents of the lumen in the unstimulated tubules also contained 20–30 mmol of phosphorus.

Our measurements on the fluid in the lumen confirm the validity of the technique. For stimulated tubules, the results for Na, K and Cl are in reasonable agreement with the findings of earlier physiological studies⁶. The fluid secreted by unstimulated *Rhodnius* tubules had not been analysed previously, but parallel isotopic measurements gave values of 50 mM for Na and 140 mM for Cl, again in good agreement. The unstimulated secretion is remarkably similar in composition to the product of the relatively slowly secreting Malpighian tubules of other insects, such as *Calliphora*^{8,11}.

The discovery of increased Na levels within the stimulated secretory cells should be considered together with the concurrent elevation of Na in the lumen. The finding is in accord with parallel measurements with ²²Na and ⁴²K, which show that in stimulated tubules the ionic composition of the secreted fluid is closely dependent on that within the cells (S.H.P.M., J. L. Wood and W. R. Harvey, in preparation). To further interpret the data we must take into account a special feature of the *Rhodnius* system: whereas the rate of fluid secretion by most insect Malpighian tubules depends strongly on the concentration of potassium ions in the bathing medium⁸, stimulated *Rhodnius* tubules can maintain high rates of secretion of Na-rich fluid in K-free media. All the available data seem to suggest that in *Rhodnius*, 5-HT (and presumably diuretic hormone *in vivo*) induces rapid entry of sodium ions into the tubule cells, and the elevation of intracellular Na is essential to a rapid secretion of sodium-rich fluid. (Similar mechanisms have been postulated for the action of hormones and neurotransmitters in several vertebrate epithelia¹²⁻¹⁶, although the

intracellular ionic changes have not been demonstrated conclusively in these tissues¹⁷.) One may postulate further that secretion is driven by an apical pump which transports Na and K in proportion to their availability.

In the brush border region, our measurements do not distinguish cleanly between microvilli and the channels between them, because the spread of a 100-nm probe in a section 1 μ m thick is too large compared with the transverse dimensions of the microvilli and the channels. The presence of mitochondria in the microvilli also adds to the scatter in the results. But the observed distributions should be qualitatively valid, and they bear directly on the way in which the movement of water may be coupled to an active movement of ions to maintain near isotonicity of the transported fluids in various animal epithelia. Among several models^{5,12}, the "standing gradient osmotic flow" hypothesis¹⁸ has been applied widely to both vertebrate and invertebrate tissues², but support¹⁸ and criticism^{19,20} of this model have been based on theory alone, in the absence of direct measurements of the gradients. In Malpighian tubules it has been suggested that the cytoplasmic processes in the region of the basal infolds and/or the extracellular spaces between the microvilli in the brush border constitute the forwardly-directed channels in which standing gradients are generated⁸. Our observations show that in *Rhodnius*, transport across the tubule brush border is unlikely to be explained in this way since the direction of the observed gradients is the reverse of that required by the model.

The sum of the Na and K levels in the brush border region is well above the Cl level. This "anion deficit" may

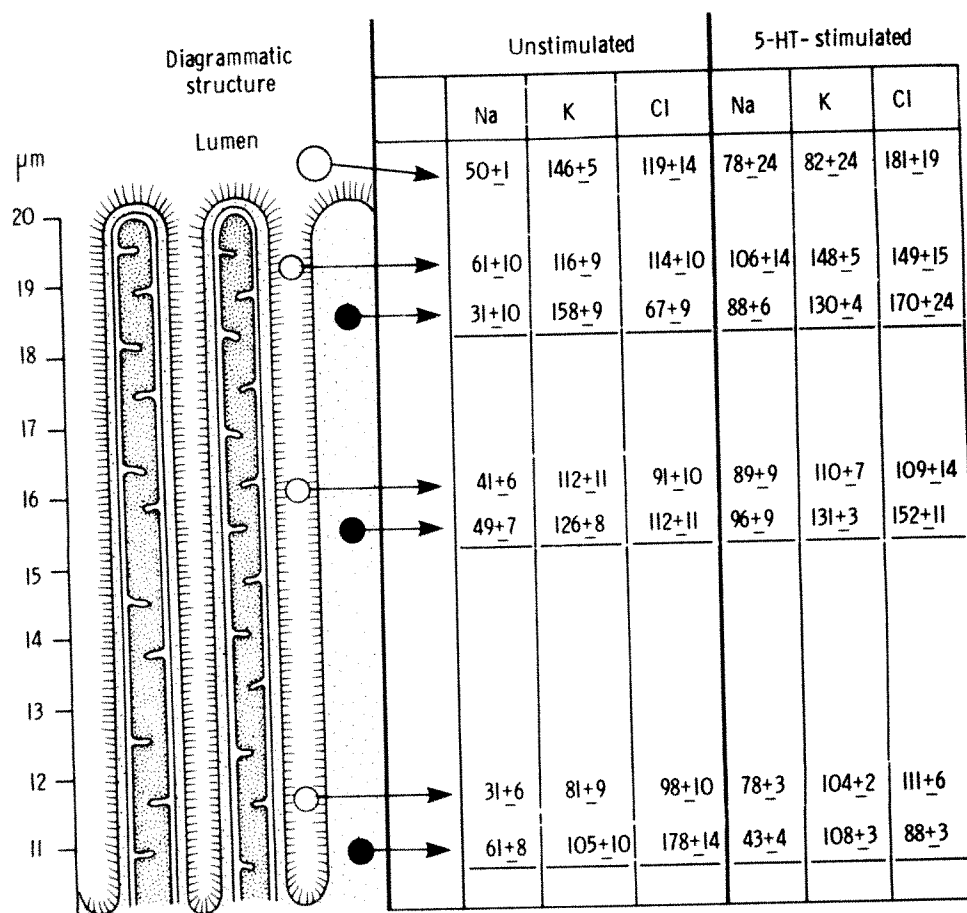


Fig. 3 As Fig. 2, but showing detailed distribution of Na, K and Cl in the microvillar region. The mitochondrion has been omitted from the marked microvilli, for clarity.

be taken as evidence of ion binding to extracellular organic material, possibly the anionic glycocalyx demonstrated by electron microscopy in other tissues^{21,22}.

In the brush border region, we need microanalyses with better spatial resolution, to be obtained with thinner sections of tissue, and we need information about the proportion of ions bound and the amount of free water present. Further experiments are in progress.

In the basal lamina, the high concentrations of potassium have now been found in other insect tissues as well (our unpublished work). The observation cannot be attributed to the limited analytical spatial resolution, which can only tend to obscure existing sharp peaks in concentration. The basal lamina has generally been regarded to be insignificant in relation to fluid transport, as experiments have shown it to be freely permeable to uncharged macromolecules²³ and colloidal particles²⁴. But our results suggest a preferential binding of K by the basal lamina, possibly to anionic sites on collagen or acidic proteoglycans²⁵. The presence of phosphoglycans is suggested by the high concentration of phosphorus, and of anionic sites by the fact that the basal lamina selectively binds lanthanum (unpublished work of B. L. G.). The implication for fluid transport is that the basal lamina, although apparently permeable to quite large neutral molecules, may nevertheless preferentially restrict the movement of some ions. Particularly in the presence of the large net fluxes of ions and water, observed in stimulated Malpighian tubules, the result may be a significant difference in composition between the external medium and that bathing the cells.

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Comparison of the biological activities of an insect and a crustacean neurohormone that are structurally similar

THE molecular structure of locust adipokinetic hormone (AKH) (ref. 1), closely resembles that of the only other arthropod neurohormone to be fully characterised, prawn red-pigment-concentrating hormone (RPCH) (ref. 2). The first eight residues of the decapeptide, AKH, are homologous with the octapeptide, RPCH, except for threonine instead of serine as residue 5, and asparagine instead of glycine as residue 7, as shown below.

RPCH: PCA-Leu-Asn-Phe-Ser-Pro-Gly-Trp-NH₂

1 2 3 4 5 6 7 8

AKH: PCA-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH₂

1 2 3 4 5 6 7 8 9 10

Although both hormones are the products of neuroendocrine organs, such close similarities in structure were somewhat unexpected, as the two hormones have completely different physiological actions. AKH in the locust controls lipid utilisation for energy during prolonged flights, by stimulating the release of diglycerides from the fat body³, and regulating their utilisation by the flight muscle⁴. RPCH in the prawn stimulates the concentration of pigment within the red chromatophores⁵. We present here evidence that the two hormones can reproduce each other's effects when cross tested on members of the two arthropod groups.

Earlier work^{6,7} had demonstrated that extracts of corpora cardiaca from several insects were able to concentrate the red chromatophore pigments in prawns and crayfish. In this preliminary study we have investigated the effects on pigment

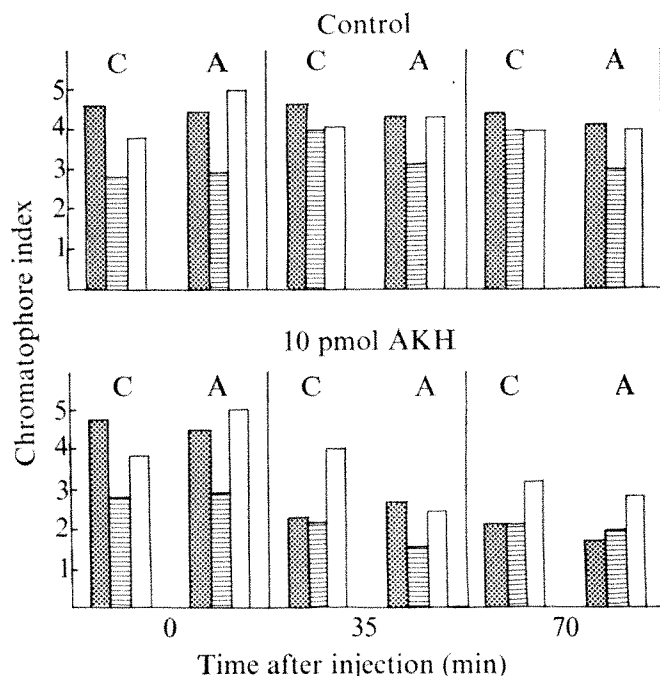


Fig. 1 Effects of AKH on the chromatophores of *Palaemon squilla*. The prawns were kept in seawater on a black background. Samples of 10 pmol AKH in 10 μ l 2.7% NaCl were injected into six animals; five control animals were not injected but were kept in identical conditions. The average chromatophore indices from groups of 20 chromatophores of each type on the carapace (C), and on the margin of the fifth abdominal sclerite (A) were estimated for each animal, and the results from each animal averaged. Stippled columns, red chromatophores; hatched columns, black chromatophores; open columns, yellow and white chromatophores.

Table 1 Effects of *Dichelopandalus* eyestalk extract and AKH on red chromatophores of *Dichelopandalus bonnierii*

Sample injected	No. of animals	Chromatophore index at various times after injection			
		0 min	20 min	45 min	100 min
Saline	4	4.8	4.9	4.8	4.6
Extract of 0.5 <i>Dichelopandalus</i> eyestalk	5	4.8	1.8		2.8
AKH : 2.0	4	4.8	2.8	1.7	3.8
(pmol) 1.0	4	4.8	Slight effect		
0.2	4	4.9	No effect		

Intact *Dichelopandalus bonnierii* kept in seawater on a red background were injected with 25 μ l 2.7% NaCl in which the test samples were dissolved. Each prawn was approximately 4 g wet weight. The chromatophore indices⁸ for groups of 20 red chromatophores on the carapace and abdomen of each animal were estimated, and the values for each animal averaged. Extracts were prepared, immediately before injection, by disrupting the eyestalks in 2.7% NaCl.

concentration of injecting pure AKH (ref. 1) into different crustacean species. We have also examined the ability of prawn eyestalk extracts to mobilise lipids in locusts. The limited supply of prawns available to us necessitated the use of extracts of eyestalks, rather than pure RPCH.

Table 1 shows the effects on red pigment concentration of injecting both a saline extract of *Dichelopandalus bonnierii* eyestalks, and AKH, into intact *Dichelopandalus*. The prawns were kept on a red background throughout the experiment, so that before injection the red pigment in the chromatophores was fully dispersed. The eyestalk extract and AKH both concentrate the red pigment markedly. Similar blanching effects were observed with methanolic extracts of *Dichelopandalus* eyestalks. When injected with 10 pmol AKH the prawns blanched completely within 5 min, whereas with 2 pmol the maximum effect was observed after 45 min. 1 pmol AKH is required to achieve some blanching in intact *Dichelopandalus*. *Pandalus montagui* also blanched slightly with this level of hormone, though neither species responded to 0.2 pmol AKH. In *Dichelopandalus* with ligatured eyestalks, and thus with the red pigment fully dispersed, three out of five animals blanched slightly with 0.2 pmol AKH; all five animals showed marked blanching 10 min after the injection of 1 pmol AKH. Hence the 'threshold' dose of AKH necessary to achieve an effect in these prawns is between 0.2 and 1 pmol.

The effects on pigment concentration of injecting AKH into

dark-adapted *Palaemon squilla* are illustrated in Fig. 1. Pigment dispersion in the red, black, yellow and white chromatophores of both the carapace and abdomen was measured. 10 pmol AKH concentrated the red and black pigments markedly and had a lesser effect on the yellow and white pigments. The effect was greatest 1 h after injection; after 2.5 h the pigments were fully dispersed again. These results may be compared with earlier studies⁹ on the effects of pure RPCH from *Pandalus borealis* on *Palaemon adspersus* chromatophores. 0.05 pmol RPCH concentrated both red and white pigments though the maximum white chromatophore response was smaller (chromatophore index (CI) 2.5) than the red chromatophore response (CI 1.0).

Dark-adapted *Crangon crangon* injected with 10 pmol AKH became very pale within 5 min of injection. The chromatophore indices of the red and black body chromatophores, and the red chromatophores on the telson, all decreased from CI 4–5 to CI 1.0 in this time and these effects persisted for 45 min. The white and yellow body chromatophores (CI 3.0) were unaffected during this time as were the black chromatophores on the telson (CI 4.5). The dispersions of black pigment in the body and telson are controlled by different factors¹⁰; our findings suggest that AKH is mimicking the body-concentrating factor.

The adipokinetic activities of methanolic extracts of eyestalks from *Dichelopandalus* and *Palaemon* injected into *Locusta migratoria* were measured using the procedure described earlier. The dose-response curves (Fig. 2) demonstrate that both extracts are able to mobilise lipids in locusts. The maximum response elicited by the *Dichelopandalus* extract was lower (11 units per locust) than that produced by the *Palaemon* extract (20 units). The maximum *Palaemon* response is, however, comparable with the maximum response of AKH (20 units per locust)¹.

We have shown that methanolic extracts of *Dichelopandalus*

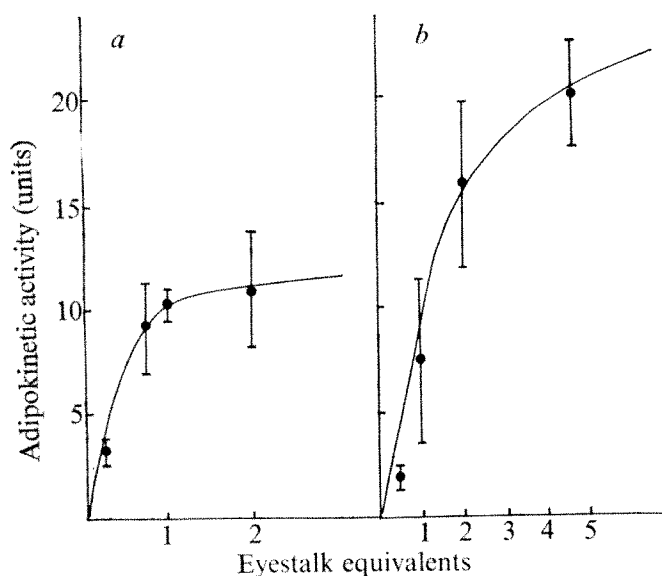


Fig. 2 Dose-response curves for the adipokinetic activity of methanolic extracts of eyestalks from *Dichelopandalus bonnierii* (a) and *Palaemon squilla* (b). The extracts, redissolved in 20 μ l simple saline were injected into adult male *Locusta migratoria* 19–21 d old and adipokinetic activities were measured as before¹. The results represent the mean activity \pm s.e. from six locusts for the *Dichelopandalus* extract and the mean activity \pm s.e. from four locusts for the *Palaemon* extract.

Table 2 Effects of thermolysin digestion on the adipokinetic activity of AKH and methanolic extracts from *Dichelopandalus* and *Palaemon*

Sample injected	Adipokinetic activity (units) of sample		Comparison of means
	Undigested	Digested with thermolysin	
Simple saline	1.1 \pm 0.7	2.1 \pm 0.4	
5 pmol AKH	19.3 \pm 2.1	2.5 \pm 1.4	$P < 0.001$
Extract from 1.5 <i>Dichelopandalus</i> eyestalks	9.5 \pm 0.4	2.8 \pm 0.4	$P < 0.001$
Extract from 1.5 <i>Palaemon</i> eyestalks	9.1 \pm 0.7	1.8 \pm 0.4	$P < 0.001$

1 nmol AKH, methanolic extracts from eight *Dichelopandalus* eyestalks, and from eight *Palaemon* eyestalks, were each dissolved in 30 μ l 0.067 M NH_4HCO_3 containing 0.15 μ mol CaCl_2 and 0.1 nmol thermolysin. The solutions were incubated for 4 h at 37 $^\circ\text{C}$, heated to 100 $^\circ\text{C}$ for 5 min and NH_4HCO_3 and water removed by lyophilisation. Identical samples, with no enzyme, were treated in the same way. The residues, redissolved in simple saline were assayed for adipokinetic activity¹ in adult male *Locusta* 16–19 d old. The results represent the mean activity \pm s.e. from four locusts.

eyestalks contain a factor with red pigment-concentrating activity. However, to establish whether the lipid-mobilising activity of these extracts is attributable to RPCH, we carried out further experiments. Both AKH¹ and RPCH² are cleaved by thermolysin; this cleavage of AKH destroys all its adipokinetic activity. Table 2 shows that thermolytic digestion of the eyestalk extracts destroys their adipokinetic activities. In addition, both the *Dichelopandalus* and *Palaemon* extracts elicited marked hyperglycaemic activity when injected into cockroaches (W.M. and J.V.S., unpublished) as does AKH (Jones, J. V. S., and W.M., in preparation). From these observations, we can conclude that the factor with adipokinetic activity present in the prawn eyestalk extracts is either RPCH or a peptide which closely resembles RPCH and AKH.

Thus locust AKH can stimulate pigment concentration in prawns and shrimps, and a peptide from crustacean eyestalks, probably RPCH, can mobilise lipids in locusts. As no pure RPCH was available, we were unable to estimate the relative potencies of RPCH and AKH in these two systems. Our findings suggest that these molecules are sufficiently similar in structure to fit on to the hormone receptors in either system; how good each 'fit' is may be indicated by comparing the molar activities of the peptides in each system. We hope to carry out such experiments in the near future.

Up to this point, our detailed knowledge of the comparative endocrinology of arthropods has been restricted to the control of moulting. The moulting hormone, 20-hydroxyecdysone, is of common occurrence in arthropods and acts on the same target, namely the epidermis, in different groups. Our results suggest that the evolution of neurosecretory hormone systems in arthropods is of a different pattern. It seems there has been a similar evolution of hormone structures, but an independent evolution of the target tissues. Examples of structurally similar peptide hormones acting on different targets in different classes are well documented among the vertebrates¹¹.

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Likely pre-Suez occurrence of a Red Sea fish *Aphanius dispar* in the Mediterranean

THE construction of artificial waterways connecting different faunal provinces provides biologists with an opportunity to observe ecological, biogeographic and evolutionary changes. But recognition of change requires a thorough knowledge of conditions before construction. The opening of the Suez Canal in 1857 is a case in point. More than thirty species of Red Sea fishes have been recorded as

colonising the Mediterranean since the opening of the canal¹, but because no reliable systematic ichthyological collections were made in the eastern Mediterranean before 1857, these new records must be judged with caution. We report here comparative electrophoretic evidence which suggests that the Red Sea cyprinodontid, *Aphanius dispar*, first reported along the Israeli coast in 1947 (ref. 2) and therefore considered a Suez migrant, has been a permanent Mediterranean resident for a long time.

A. dispar (Rüppell) is a small, littoral, euryhaline fish widely distributed in the Indian Ocean, which also occurs along both coasts of the Sinai Peninsula and in the Suez Canal. In the Mediterranean, it was reported at Port Said³ and subsequently along the Israeli coast from Atlit, Tel Aviv, and Caesarea^{2,4}. In addition, several subspecifically distinct populations (*A. dispar richardsoni* (Boulenger)) occur as isolates in freshwater pools along the periphery of the Dead Sea. These Dead Sea fishes have been living in isolation since the early Pleistocene⁵ and differ from conspecifics in male breeding coloration⁶. But all populations of the species have the same gross karyotype, cross freely in aquaria and are morphologically indistinguishable^{6,7}.

We collected population samples of *A. dispar* at five localities from the Mediterranean, Red Sea and Dead Sea (Fig. 1). All specimens were examined for genetic variability at nineteen putative isoenzyme loci by standard methods of starch gel electrophoresis⁸ (Table 1). Genetic similarity between all possible pairs of these populations was calculated by means of Rogers' coefficient (S_R); the values are provided in Table 2.

In general, excluding comparisons between subspecies and karyotypically differentiated populations (which could easily be regarded as sibling species), virtually all intra-specific estimates of genetic similarity (S_R) in a wide variety of organisms have been greater than 0.75^{10,11}. As expected, both population pairs within the Red Sea and Dead Sea exhibit a very high degree of similarity. The estimates of similarity for *A. dispar* between the Mediterranean, Red Sea and Dead Sea are in sharp contrast, however (Table 2). Because *A. dispar* was presumed to have entered the Mediterranean only recently through the Suez Canal^{12,13}, it was expected that this population sample would approximate the level of divergence observed among Red Sea samples. Instead, the degree of difference between Mediterranean and Red Sea samples is as large as that between either sample

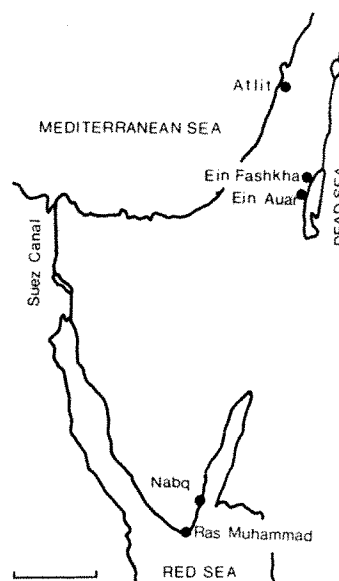


Fig. 1 Sampling localities of *Aphanius dispar* in Israel. The scale equals 100 km.

Table 1 Genetic variation at 19 loci in five populations of *A. dispar*

Sample area	Locality	Heterozygosity*	Average no. of alleles per locus	Unique alleles†
Mediterranean	Atlit	6.27	1.26	6
Red Sea	Nabq	2.39	1.37	8
Red Sea	Ras Muhammad	6.00	1.26	
Dead Sea	Ein Fashkha	5.10	1.26	6
Dead Sea	Ein el Ghuweir	4.56	1.11	

Thirty individuals were examined from each locality. With the exception of the assay for lactate dehydrogenase (LDH), all proteins were examined from whole-body homogenates diluted 1:1 with deionised water. LDH was examined from individual homogenates of eye. Electrophoretic procedures and histochemical staining were similar to those described before⁸. Specimens were examined for the following proteins (number of presumed loci in parentheses): esterase (2), LDH (3), malate dehydrogenase (2), phosphoglucosutase (3), isocitrate dehydrogenase (1), general protein (2), α -glycerophosphate dehydrogenase (1), 6-phosphogluconate dehydrogenase (1), phosphoglucose isomerase (2), aldolase (1), and amino peptidase (1).

*Heterozygosity was calculated as the average observed number of heterozygotes per locus, expressed ($\times 100$).

†Unique alleles are those alleles which were observed in only one out of the three sample areas.

and the Dead Sea isolates, whose last conspecific contact was during the early Pleistocene. Such genetic differences are of the order normally associated with interspecific comparisons, where long periods of isolation are indicated¹³. We conclude therefore that *Aphanius dispar* was extant in the Mediterranean before the construction of the Suez Canal.

Our conclusion reinforces speculations about the pre-Suez occurrence of *A. dispar* in the Mediterranean. This species has previously^{14,15} been considered a likely candidate for pre-Suez migration because of its wide salinity tolerance and the high probability of early interoceanic contact facilitated by ancient channelling¹⁶ and eustatic fluctuations.

A. dispar prevented survival in a cooling Mediterranean¹², but measurements of thermal tolerance (our unpublished results) and recent palaeoclimatological estimates of the Pleistocene Mediterranean²⁰ invalidate such concern. There is no support for an extinction of this population. A further argument is by analogy to the occurrence of a congeneric species, *Aphanius fasciatus*. This fish is a widely distributed Mediterranean endemic common in the littoral. After the opening of the Suez Canal, it was reported in abundance from Lake Timsah, 75 km south of the Mediterranean, yet it was formally recorded from the Israeli coast only in 1954¹⁸. Thus, if an ecologically similar and widely distributed Mediterranean resident was not detected along the Israeli

Table 2 Genetic similarity (S_R)⁹ among five Israeli populations of *A. dispar*

	Mediterranean Sea Atlit	Nabq	Red Sea Ras Muhammad	Ein Fashkha	Dead Sea Ein el Ghuweir
Atlit	—	0.721	0.716	0.596	0.580
Nabq		—	0.972	0.728	0.708
Ras Muhammad			—	0.731	0.721
Ein Fashkha				—	0.979
Ein el Ghuweir					—

At least two alternative explanations for our observations, consistent with the idea of recent Suez migration, merit attention. First, stochastic events associated with colonisation may have reduced the level of variability and provided a biased sample of founding Red Sea genes. Although a normal level of heterozygosity could easily become re-established if population size increased rapidly, the average number of alleles per locus would recover much more slowly from a severe bottleneck¹⁷. The Mediterranean sample, however, is not distinguished by either homozygosity or a lower average number of alleles compared with conspecifics (Table 1). More convincingly, the presence of unique alleles in all areas sampled (Table 1) can be taken as evidence of substantial isolation and absence of gene flow. Second, the source material for the Mediterranean colonisation may not have come from the Red Sea itself, but from some geographical isolate within the Suez area. For example, the founders could have come from a genetically differentiated population isolated in the Great Bitter Lakes. While this suggestion cannot be excluded, we consider it unlikely (see below).

Several further arguments support our contention that *A. dispar* is of pre-Suez occurrence in the Mediterranean. The progenitors of the Dead Sea subspecies must have colonised the Jordan drainage system from the Mediterranean^{18,19}. The idea that the extant population of *A. dispar* in the Mediterranean is of recent origin is associated with the assumption that the original founding population became extinct. It has been argued that the thermophilic nature of

coast until very recently, what degree of confidence can be placed in early negative reports for *A. dispar*?

Our findings have implications for future oceanic waterways. Clearly, only extremely comprehensive systematic surveys can provide the necessary baselines for evaluating the impact of major faunal interchange. We follow many others^{21,22}, in reiterating this concern with respect to the proposed and much debated sea level canal in Panama.

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Chromosomal recombination and mapping in *Rhizobium leguminosarum*

IGNORANCE of the genetics of the nitrogen-fixing symbiosis between *Rhizobium* species and leguminous plants is due chiefly to the lack of a good system for genetic mapping in *Rhizobium*. Although gene transfer by transformation and transduction have been reported in several *Rhizobium*

species^{1,2}, the only linkage map has come from studies of conjugation in a non-nodulating *R. lupini* strain³ and a genetic analysis of symbiotically defective mutants has not been possible. *R. leguminosarum* is particularly suitable for genetic studies since it nodulates the pea (*Pisum sativum*), a genetically well known legume^{4,5}. Initial studies with many *R. leguminosarum* wild types failed to demonstrate recombination⁶ although P group R factors could be transferred between them^{6,7}. These plasmids have wide host ranges⁸ and have been shown to mediate the transfer of chromosomal genes in *Pseudomonas*⁹, *Escherichia*¹⁰ and *Acinetobacter*¹¹. R68.45 is a derivative of the P group R factor (R68) isolated by Haas and Holloway⁹ for having much better sex factor activity in *P. aeruginosa* than R68 and other R factors. We report here that it behaves in a similar manner in nodulating strains of *R. leguminosarum* and can be used for genetic mapping in this species.

Table 1 Analysis of four-factor crosses: *phe-1 ser-1* (R68.45) × *ura-14 met-14 rif-74* (cross A) and *phe-1 ser-1* (R68.45) × *ura-14 trp-16* (cross B)

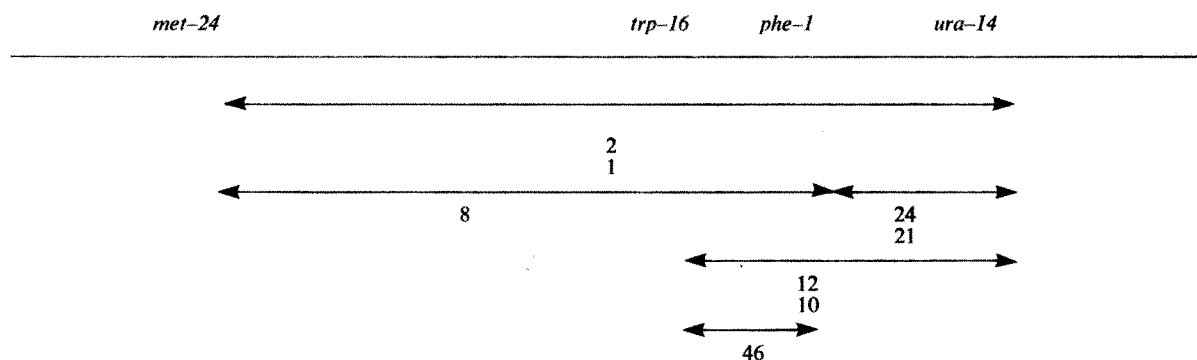
(a) Genotypes of recombinants from selective media

Genotypes of selectable progeny				No. of recombinants of each genotype on selective media supplemented with					Average frequency
Cross A	<i>phe</i>	<i>ura</i>	<i>ser</i>	<i>met</i>	Phenylalanine + uracil	Phenylalanine + methionine	Serine + uracil	Serine + methionine	
	+	+	+	+	1	0	0	1	1
	+	+	+	+	1	1	—	—	1
	+	+	+	+	124	—	133	—	129
	+	+	+	+	—	—	0	0	0
	+	+	+	+	—	109	—	117	113
	+	+	+	+	10	—	—	—	10
	+	+	+	+	—	—	—	0	0
	+	+	+	+	—	33	—	—	33
	+	+	+	+	—	—	0	—	0
Cross B	<i>phe</i>	<i>ura</i>	<i>ser</i>	<i>trp</i>	Phenylalanine + uracil	Phenylalanine + tryptophan	Serine + uracil	Serine + tryptophan	Average frequency
	+	+	+	+	1	7	2	0	3
	+	+	+	+	22	13	—	—	18
	+	+	+	+	102	—	185	—	144
	+	+	+	+	—	—	0	1	1
	+	+	+	+	—	148	—	192	170
	+	+	+	+	67	—	—	—	67
	+	+	+	+	—	—	—	0	0
	+	+	+	+	—	27	—	—	27
	+	+	+	+	—	—	0	—	0

(b) Analysis of the frequencies of cotransfer of selected and non-selected markers

	Phenylalanine + uracil	Phenylalanine + methionine or tryptophan	Serine + uracil	Serine + methionine or tryptophan
Cross A				
Counterselected marker	(<i>ser</i>)	(<i>ser</i>)	(<i>phe</i>)	(<i>phe</i>)
Selected marker	<i>met</i> ⁺	<i>ura</i> ⁺	<i>met</i> ⁺	<i>ura</i> ⁺
Non-selected markers	<i>ura</i> ⁺ : 2/136 (2%) <i>phe</i> : 11/136 (8%)	<i>met</i> ⁺ : 1/143 (1%) <i>phe</i> : 34/143 (24%)	<i>ura</i> ⁺ : 0/133 <i>ser</i> : 0/133	<i>met</i> ⁺ : 1/118 <i>ser</i> : 0/118
Cross B				
Counterselected marker	(<i>ser</i>)	(<i>ser</i>)	(<i>phe</i>)	(<i>phe</i>)
Selected marker	<i>trp</i> ⁺	<i>ura</i> ⁺	<i>trp</i> ⁺	<i>ura</i> ⁺
Non-selected markers	<i>ura</i> ⁺ : 23/192 (12%) <i>phe</i> : 89/192 (46%)	<i>trp</i> ⁺ : 20/195 (10%) <i>phe</i> : 40/195 (21%)	<i>ura</i> ⁺ : 2/187 <i>ser</i> : 0/187	<i>trp</i> ⁺ : 1/193 <i>ser</i> : 1/193

(c) Mapping of mutations by co-transfer percentages



For both crosses the mating mixture was plated undiluted on the four selective media; colony counts on the four media were approximately equal. 150 colonies from cross A and 200 from cross B were picked from each selective medium and characterised for non-selected markers.

We tested RP4, which has been used for conjugation studies in *R. leguminosarum*⁷, R68.44 and R68.45 for their ability to promote recombination in crosses between *R. leguminosarum* strains, using a membrane mating procedure¹². In these crosses equal numbers of donor and recipient bacteria were sucked on to Millipore membranes and incubated overnight on complete medium plates. The bacteria were then washed off in distilled water and between 10⁹ and 10¹⁰ bacteria were plated on each selective medium. The transfer frequencies of RP4, R68.44 and R68.45 (per recipient) were about 10⁻². The R factors were introduced into the *R. leguminosarum* strains by crossing them with appropriate R⁺ *E. coli* donors¹². All *R. leguminosarum* strains were derivatives of the wild-type strain 300¹³ which is able to form normal nitrogen-fixing nodules on the pea (variety Wisconsin Perfection).

RP4 and R68.44 were equally inefficient in promoting chromosome transfer in *R. leguminosarum*. For both, the recombination frequency of approximately 10⁻⁹ per recipient was similar to the reversion rates of the markers being examined and was quite unsuitable for mapping purposes, though a low frequency of true recombination was proved by the recovery of some progeny carrying auxotrophic markers of both parents¹². R68.45 was about 100 times more efficient and crosses using it routinely produced enough recombinants for linkage analysis. Four-factor crosses of the type described by Hopwood^{14,15} for mapping in *Streptomyces* were used because they facilitate recovery of nine of the 16 possible combinations of parental markers by plating on four different selective media and therefore provide a considerable amount of information.

In such crosses, two of which are shown in Table 1a, four of the recovered genotypes differ from one parent or the other by a single marker and the other five differ from both parents by two markers. Of these five, four (the last four in each tabulation) represent two pairs of complementary genotypes. It is clear that: (1) the genotypes differing from

Table 2 Segregation of *rif* and *str*

Cross B	No. of R ⁺ colonies selected on medium containing phenylalanine, cysteine, tryptophan and rifampicin	
<i>ura-14 met-14 rif-74</i> (R68.45)	<i>phe trp rif str</i>	<i>phe trp rif</i>
×	2	65
<i>phe-1 trp-12 str-37</i>		
Cross C	<i>phe cys rif str</i>	<i>phe cys rif</i>
<i>ura-14 met-14 rif-74</i> (R68.45)		
×	2	86
<i>phe-1 cys-7 str-77</i>		

Cross mixtures were plated undiluted and all colonies were picked and characterised for non-selected markers. R⁻ *phe cys str rif* and *phe trp str rif* colonies were assumed to be spontaneous mutants; there were 12 for cross B and none for cross C.

the R⁻ parent by a single marker were much more frequent than those differing from the R⁺ parent by a single marker; (2) one member of each complementary pair of genotypes was much more frequent than the other. Thus we conclude that, as with F⁺ × F⁻ or R⁺ × R⁻ crosses in *E. coli*, transfer was polarised from the plasmid-carrying parent and we can analyse the data for linkage on this assumption. On each plating medium, one donor marker is selected, one is counterselected and the other two are non-selected. The frequencies of the donor non-selected alleles are tabulated (Table 1b). There is no significant transfer of *ser-1*, which is therefore unlinked with the other four; therefore we can analyse their linkage on the media on which *ser-1* is counterselected, without complications due to their possible linkage with the counterselected allele. The other four markers show linkage and the percentage cotransfer frequencies indicate a probable map order *met-14-trp-16-phe-1-ura-14* (Table 1c).

Segregation of *str* and *rif* as non-selected markers in further crosses indicated that neither was closely linked to *ura-14*, *met-14* or *phe-1* and that they appeared to be linked together. A selective analysis of their segregation showed that they were closely linked: selection for transfer of *rif* to the R⁻ parent nearly always led to loss of the recipient *str* marker (Table 2). In *P. aeruginosa*¹⁶, *Bacillus subtilis*¹⁷ and *Streptomyces coelicolor*¹⁸, *rif* and *str* genes are also closely linked; in the latter two organisms rifampicin resistance mutations mapping near *str* have been shown to be in the RNA polymerase gene^{17,18}.

The mutation *trp-16* is closely linked to *phe-1* (Table 1) but *trp-12* showed no linkage with *met-14*, *phe-1* or *ura-14*; this indicates that, unlike *E. coli* or *Bacillus subtilis*, but like *S. coelicolor*¹⁹ and *P. aeruginosa*²⁰, *R. leguminosarum* does not have a single tryptophan operon. This observation is confirmed by data from crosses involving a *R. leguminosarum* donor carrying a derivative of RP4 that mediates the transfer of *trp⁺-14* about 100 times more frequently than RP4 (J.E.B., unpublished results). This donor transfers *trp⁺-14* and *trp⁺-12* at the same high frequency, but *trp⁺-16* at the frequency expected for a wild-type RP4 donor.

In crosses between *P. aeruginosa* strains, R68.45 has multiple sites of origin of chromosome transfer and mobilises fairly short fragments of donor chromosome (about 10–30 min of the *P. aeruginosa* map)⁹. Both these properties of the plasmid seem to apply in *R. leguminosarum* crosses. When selection is for the transfer of markers not showing linkage with *phe-1*, such as *str-37* or *rif-74*, they are found to be transferred as frequently as *phe⁺-1*. Analysis of recombinants shows that, while fairly long fragments can be transferred, most recombinants arise from the inheritance of smaller donor fragments (Table 1). R68.45 transfers at a lower frequency between *R. leguminosarum* strains than between *P. aeruginosa* strains (about 1% and 50% respectively) and this is correlated with the lower frequency of recombination observed in *R. leguminosarum*. But the problem of distinguishing spontaneous revertants from recombinants when analysing progeny on selective media was facilitated by the low frequency of R factor transfer. Spontaneous revertants have a 1% chance of becoming R⁺ and therefore if true recombinants are being formed (for markers with suitably low reversion rates) they should be R⁺. Routinely this was observed; fewer than 5% of colonies on selective media were R⁻.

Why is R68.45 able to mobilise *P. aeruginosa* and *R. leguminosarum* chromosomal genes so efficiently when RP4 and R68.44 are so inefficient in *R. leguminosarum*? RP4 (RP1, R1822)²¹ is a poor sex factor in *P. aeruginosa*⁹ but a good one in *Acinetobacter*¹¹. The donor properties of this plasmid are therefore strain (or species) specific and *R. leguminosarum* strain 300 behaves like *P. aeruginosa* strains PAO and PAT. R68.44 would be expected to behave like R68.45 in *R. leguminosarum*. However R68.44 is an unstable donor in *P. aeruginosa*⁹ and presumably lost this property during its transfer from *P. aeruginosa* to *E. coli* and then to *R. leguminosarum*. R68.44 and a number of similar plasmids were isolated by testing *argB⁺* recombinants in *P. aeruginosa* for enhanced donor activity⁹. The testing of recombinants arising from the transfer of markers from other regions of the chromosome did not yield such donors. Therefore it is likely that R68.44 (and hence R68.45) arose as the result of an interaction between the R factor and *P. aeruginosa* chromosome, rather than the selection of an already existing plasmid mutant. It seems unlikely that R68.45 functions as an improved sex factor in *P. aeruginosa* by virtue of being analogous to an F-prime in *E. coli*. If it were an R-prime it should only have one origin of high frequency transfer in *P. aeruginosa* and would not be expected to function in *R. leguminosarum*. R68.45 also promotes chromosome transfer in *E. coli* K12 strains more

efficiently than R68 (J.E.B. unpublished observations). It seems likely therefore that R68.45 was formed by the pick-up of a *P. aeruginosa* DNA sequence(s) that enables it to interact with the chromosomes of genetically distinct hosts. Insertion sequences are involved in "illegitimate" recombination events^{22,23} and may well be implicated here. Since R68.45 can function as a good sex factor in *R. leguminosarum*, *P. aeruginosa*, *P. putida* and *E. coli* it may be useful in developing genetic studies in other Gram-negative bacteria that are hosts for P group R factors.

We hope that development of the recombination system described here will open the way to a genetic analysis of mutations affecting the nitrogen-fixing symbiosis between *R. leguminosarum* and the pea. It is significant that the majority of the auxotrophic and antibiotic-resistant mutations used as markers did not prevent the formation of effective nodules by multiply marked R⁺ or R⁻ strains (J.E.B., unpublished results) and so should not interfere with the study of relevant mutations.

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Note added in proof: Since completing this work, we have learnt that Meade and Signer²⁴ have demonstrated linkage between chromosomal genes in *R. meliloti* strain 2011, using RP4 as a sex factor.

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Lack of correlation between tight junction morphology and permeability properties in developing choroid plexus

ELECTRON microscopical investigations of tight junctions (zonulae occludentes) using marker substances, have indicated that in some tissues these junctions may represent an extracellular pathway for transepithelial movement of certain small solutes¹ and also restrict the movement of larger solutes². The electrical resistance across some epithelial tissues has been used as a measure of the degree of permeability of the tight junctions to ions³, and it was suggested that junctions that are "leaky" to ions are also leaky

to small non-electrolytes. Examination of tight junctions with the freeze-fracture technique has revealed the presence of a network of intramembranous fibrils⁴ or strands^{5,6}. Some evidence suggests that these strands constitute the sealing component of the junction⁷, and Claude and Goodenough⁸ correlated the transepithelial permeability of a given epithelium with the number of strands in the tight junction network. Artificial methods of altering transepithelial permeability (hypertonic solutions applied to the mucosal side of the epithelium) have been used to investigate changes in transepithelial electrical resistance⁹, and freeze-fracture morphology⁹ or both¹⁰. Results from these studies do not consistently support the above correlation. An alternative approach is described here, in which the freeze-fracture technique has been used to study tight junctions of the sheep choroid plexus during foetal development. The results obtained suggest that there is no change in the ultrastructural features of tight junctions which have previously been suggested to correlate with transepithelial permeability, in spite of considerable changes in permeability during the developmental period studied¹¹⁻¹³.

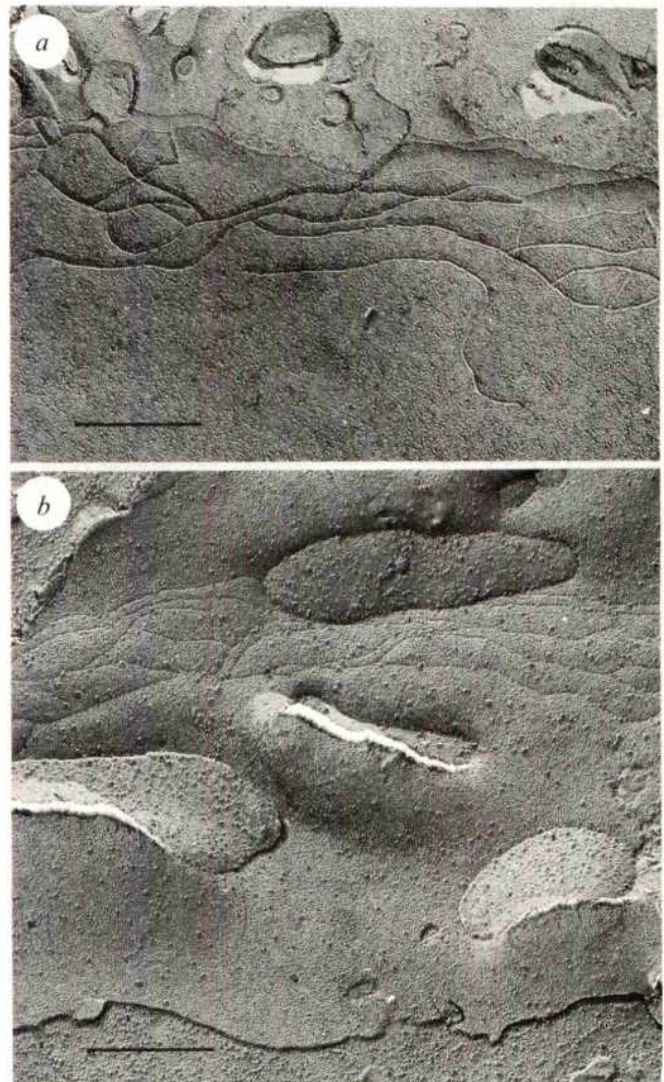


Fig. 1 Freeze-fracture replicas of a choroid plexus epithelial cell from a, a 40-d and b, a 125-d gestation foetal sheep. a, The fracture has exposed a large area of tight junction towards the apex (cerebrospinal fluid surface) of the cell. There are at least four strands running roughly parallel to the apical cell surface. b, Note the similar number of strands compared with 40-d (a) and that the junctional depth is at least as great at 40-d as at 125-d. Bar indicates 0.5 μ m.

Freeze-fracture replicas were prepared from sheep foetal choroid plexuses at 40, 45, 60 and 125 d of gestation using methods described previously¹⁴. Figure 1a shows a replica of a fracture through the apical region of the choroid plexus epithelium of an early sheep foetus (40 d gestation); it shows the cell membrane faces of adjoining cells. Below the level of the microvilli are seen the linear ridges and complementary linear grooves which constitute the tight junction. Figure 1b shows a replica at 125 d gestation; its appearance is not obviously different from that at 40 d. Similar results were obtained at 45 and 60 d gestation. To test whether permeability of non-electrolytes and establishment of ionic gradients might correlate with strand number and/or junctional depth, detailed studies of a large number of tight junctions at different foetal ages have been carried out (K.M. and N.R.S., unpublished). Measurements of the minimum number of strands and the mean junctional depth were made as described by Claude and Goodenough⁴. Freeze-fracture replicas of more than 400 areas of lateral-apical cell membrane have been examined. Even in the youngest foetuses the choroid plexus intercellular tight junctions contained several continuous strands in all cases. At 40 d gestation the minimum number of strands was 3.64 ± 1.12 and the mean junctional depth was $0.34 \pm 0.17 \mu\text{m}$ (s.d., $n=121$). At 125 d gestation the number of fibrils (3.45 ± 1.13 ; s.d., $n=49$) was not significantly different, but the junctional depth ($0.28 \pm 0.14 \mu\text{m}$; s.d., $n=49$) was significantly less. It has been shown that at these gestational ages, the thin section electron-microscopic appearance of the tight junctions was similar (C. A. N. Evans, D.H.M., K.M., J. M. Reynolds, M. L. Reynolds and N.R.S., unpublished).

Studies of the penetration of materials from blood into brain and cerebrospinal fluid (CSF) in sheep foetuses of the same gestational ages as those examined in the present study have shown considerable changes in the permeability characteristics of the system during foetal development. For example, lipid-insoluble molecules of small molecular weight (erythritol, sucrose and inulin) as well as albumin, penetrate from blood into CSF at a rate and to an extent which may suggest unrestricted passive diffusion at 60 d; in contrast, there is considerable restriction by 125 d, at which stage the mean pore radius was estimated to be about 1 nm (unpublished). Foetal development of CSF-plasma electrolyte gradients has been discussed previously¹¹ and the gradients for individual ions were shown to appear at very different stages in the order magnesium, chloride, potassium, calcium. The concentration of protein in early foetal CSF is very high, for example 500 mg per 100 ml in 55-d sheep foetuses. At least part of this high level seems to be caused by a mechanism which transports specific proteins (for example, α -foetoprotein and transferrin) from blood into CSF early in gestation but not later when the CSF concentration of protein has fallen to near adult levels (50 mg per 100 ml)¹³.

Since tight junction strand number as revealed by freeze fracture does not change significantly during a major part of gestation, this cannot account for the observed alterations in the permeability properties. From the wide variety of epithelia studied with the freeze-fracture technique it is clear that there are important exceptions to the proposed correlation of permeability with fibril (strand) number (ref. 10 and refs therein). Additional evidence against this correlation comes from recent experiments¹⁹ which showed that in the toad bladder treated with hypertonic lysine there was a marked decrease in transepithelial resistance, but no significant change in either fibril number or mean depth of the tight junctions.

It may be that some other feature of tight junctions not revealed by electron microscopy could account for some of these changes in permeability. Both the molecular structure of the junctional membrane and the biochemical composi-

tion of the material enclosed by the junction fibrils might be important for passive ion transport. Nevertheless, one type of extracellular pathway through tight junctions could not account for all our permeability data for the blood-CSF barrier. For example, as mentioned above, there is penetration of albumin from blood into CSF in the 60-d foetus. Such a large pathway, were it present within choroid plexus tight junctions, would be well within the resolution of our electron-microscopical observations. It seems more likely that protein and non-electrolyte penetration and perhaps some type of ion transport involves a quite different route, that is, a transcellular one, consisting of a tubulovesicular system. An intracellular system of tubules and cisternae of endoplasmic reticulum has recently been implicated in transcellular transport of protein¹⁴ and non-electrolytes (C. A. N. Evans, D. H. M., K.M., J. M. Reynolds, M. L. Reynolds and N.R.S., unpublished) in foetal choroid plexus as well as in active sodium transport in frog skin¹⁵.

This work was supported by grants from the Danish Medical Research Council, the Medical Research Council and the Wellcome Trust. We thank B. Lauritzen for technical assistance, P. Holstein and S. Mulbjerg for the computer analysis of the complete data and Professor Sir Andrew Huxley and Professor H. H. Ussing for reading the manuscript.

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Errata

The order of the authors of the article "Release and metabolism of substance P in rat hypothalamus" (*Nature*, **264**, 81; 1976) should have been T. Jessell, L. L. Iversen and I. Kanazawa and not as printed.

In the article "Stratospheric aerosols and climatic change" (*Nature*, **263**, 551; 1976) the order of the authors' names was changed and should have read J. B. Pollack, O. B. Toon, A. Summers, B. Baldwin, C. Sagan and W. Van Camp.

In the article "Polymers for the sustained release of proteins and other macromolecules" by R. Langer and J. Folkman (*Nature*, **263**, 797; 1976) the units on the abscissa in Fig. 1 should be hours.

matters arising

A precise definition of chaos

MAY¹ has recently reviewed work on the dynamics of simple recurrence relations. If $x_{n+1} = f(a; x_n)$ where a is a parameter, the system exhibits different behaviours for different values of a . In many cases, there is a range of behaviour characterised by three properties:

- (1) Not all solutions are periodic;
- (2) Asymptotic stability need not obtain;
- (3) Cycles of all periods may occur.

Li and Yorke² show that (1), (2) and (3) necessarily follow if a 3-cycle exists and these authors term such behaviour 'chaotic'. They also show that a 5-cycle may exist without there being a 3-cycle.

The Ukrainian author Sharkovsky³ proves a more general result, which deserves to be more widely known. Let $n_1 < n_2$ if $n_1 \neq n_2$ and the existence of an n_1 -cycle implies the existence of an n_2 -cycle. Sharkovsky shows that

$$\begin{aligned} 3 &< 5 < 7 < 9 < \dots \\ &< 2.3 < 2.5 < 2.7 \dots \\ &< 2^2.3 < 2^2.5 < 2^2.7 < \dots \\ &< 2^3.3 < 2^3.5 < 2^3.7 \\ &< \dots < \dots 2^3 < 2^2 < 2 < 1 \end{aligned} \quad (1)$$

He also demonstrates that there exist recurrence relationships with $(2m+1)$ -cycles but not with $3, 5, \dots, (2m-1)$ -cycles, for any natural number $m = 2, 3, 4, \dots$

In a subsequent paper⁴, Sharkovsky classifies the behaviour of the system in terms of the topological properties of the set C of all points belonging to cycles. If C is a closed set, then all initial values x_0 lead to asymptotically periodic behaviour. This occurs when there are only finitely many cycles (necessarily 2^n -cycles by relation (1)), and in some instances where there are infinitely many cycles all with orders of the form 2^n . If C is not a closed set the number of x_0 not leading to asymptotic periodicity is uncountably large. This occurs whenever there are cycles other than 2^n -cycles and, in some cases, where all the 2^n -cycles occur but only those.

In the first of these instances, requirements (1) and (2) are satisfied, but not necessarily (3). It is not known whether this is true in the second instance, which, however, we conjecture to be structurally unstable to changes in the value of a .

We suggest that requirements (1) and (2) be regarded as defining chaos, but that (3) not be regarded as necessary. This would entail some divergence from the nomenclature of certain authors (for

example, May¹), but it is a consistent convention and one in accord with everyday usage.

A detailed, though still incomplete, list of Sharkovsky's papers is provided by Sibirsky⁵. We also draw attention to the work of Barna⁶, Berg⁷ and Coppel⁸.

We thank Mr W. A. Coppel for his help in supplying some of the reference material. A more complete account of this work will appear elsewhere.

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Heat-transfer enhancement with electric fields

CHANGES in heat transfer brought about by electric fields, as reported by Asakawa¹, have been observed for many years. The discovery of the phenomenon is generally attributed to Senftleben and Braun² who observed the systematic enhancement of heat transfer in various gases contained between a cylindrical surface and a coaxial wire when electric fields greater than about 42 kV cm^{-1} were produced at the surface of the wire. The effect was mentioned as early as 1709, however³.

The results reported by Asakawa seem primarily to be the retardation of heat transfer, but in fact seem similar to those expected on the basis of simple inductive heating. It is difficult to quantify heat transfer results with transient experiments, and is generally more informative to rely on steady-state (for example, calorimetric) measurements. Although it is apparently tempting to suggest that the phenomena observed by Asakawa involve material

changes in the material (fluid), there is no evidence for such a conjecture.

We have studied the enhancement of heat transfer in gases by inhomogeneous electric fields in some detail⁴. Our results show that this effect, which, for convenience we call electrocooling, is simply an ionic drag phenomenon (that is, the electric wind of Chattock⁵) in which the convective heat transfer coefficient is proportional to the fourth root of the induced corona current. We derived a theoretical expression which predicts such corona-induced heat transfer within 20% of experimental values for electro-negative gases such as air. As a particular type of forced convection, electrocooling may thus be directly compared to conventional devices such as air jets or blowers.

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Scrotal asymmetry and Rodin's dyslexia

MCMANUS¹ has pointed out that most artists depict the left testicle of man lower than the right, in accordance with the facts². An exception occurs in Rodin's famous sculpture *L'Age d'Airan* where the right testicle of the figure seems (from photographs we have seen) to hang lower than the left. We should like to suggest that Rodin was genuinely confused about left and right. Evidence that Rodin suffered from a specific reading disability, which is commonly associated with left-right confusions³, has been reviewed by Thompson⁴. So badly did the artist do in school during his early years that his father was informed: "He is ineducable. The sooner you put him out to work, the better. But I doubt if he can ever make a living."⁵

Some of the facts about scrotal

asymmetry seem themselves rather confused. According to Chang *et al.*², left-handed people may show a reversal of the usual scrotal asymmetry. Cholst⁶, however, maintains that reversal occurs only in the extremely rare condition of *situs inversus totalis*. Chang *et al.* also report that the right testicle is usually heavier than the left, which seems superficially at odds with the observation that the left testicle is lower¹. The generality of this finding should perhaps be checked, in view of the puzzling discrepancy over the relationship to handedness. Nevertheless there is some support for the weight difference described by Chang *et al.* in a recent report that the right human gonad normally develops more rapidly than the left⁷.

We have recently suggested that there is a general tendency for growth gradients to favour the left-hand side^{8,9}. Examples are seen in the temporal planum of the human brain; the habenular nucleus in the brains of amphibia; the control of birdsong by the left hypoglossal nerve; the cervical vertebrae of chickens; the greatly elongated left tooth of the Narwhal; and the left-sided development of the hydrocoel in echinoderms. The concordance among these asymmetries is intriguing, but we are obliged to admit that the faster development of the right gonad in mammals tends to restore parity to the situation.

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Rosette formation by human lymphocytes

HANAUMI *et al.*¹ point out the need to elucidate the nature of the receptor for sheep erythrocytes (E) on human T lymphocytes. They have examined the effect of phospholipase A (PLA) on E-rosette formation in a study of

the specificity of the type of lymphocytes involved. They showed that PLA from cobra venom induced the ability to form E rosettes in B-type human peripheral lymphocytes and lymphoid cell lines. In contrast, PLA from bee venom and bovine pancreas were ineffective.

I have used PLA from *Crotalus adamanteus* venom, partially purified on Sephadex G-200 and assayed by a lecithin breakdown test^{2,3}. Incubation of this preparation with human lymphocytes at 37 °C for 1 h resulted in a marked decrease in E-rosette formation. Titration experiments showed that the maximum inhibitory effect was produced with PLA at 1 mg ml⁻¹. The effect of this enzyme preparation was resistant to heating to 100 °C for 10 min, but it was not active at 4 °C. It was non-toxic to human lymphocytes and erythrocytes as measured by release of radioactive chromium. Lymphocytes could recover the ability to form E rosettes ~ 8 h after removal of the enzyme on further incubation at 37 °C. Furthermore, PLA treatment of human spleen membranes released material which could block erythrocytes^{2,3}.

These earlier results conflict with those of Hanaumi *et al.*¹, but the discrepancy may be a result of the different sources of enzyme¹. In addition, Zwaal *et al.*⁴ have shown that PLA purified from cobra venom and bee venom does convert lecithin to lysolecithin, in contrast to that purified from bovine pancreas. They also showed that *in situ* lysolecithin does not cause lysis (of human red cells).

This difference between results is striking. I showed that PLA-treated lymphocytes do not form rosettes, probably because of removal of the E receptors which can be re-expressed by the lymphocytes after removal of the enzyme. This may provide a method of solubilisation of the receptors for chemical characterisation. Hanaumi *et al.* showed however, that the E receptor is resistant to their PLA preparation and suggested that there are E receptors on human B cells which are exposed by PLA, although they were unable to show that neuraminidase treatment unmasked these receptors on B-type lymphoid cell lines, in contrast to peripheral B lymphocytes⁵.

This difference has practical importance for the further study of E receptors.

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HANAUMI AND KUMAGAI REPLY—We thank Chapel for her comments¹ on our report concerned with the nature of the receptor for sheep erythrocytes on human lymphocytes and lymphoid cell lines². We too have tested the effect of PLA partially purified from the venom of *Crotalus terrificus*, which is one of the Crotalidae snakes, but not *C. adamanteus* used by Chapel³ on the lymphocytes and lymphoid cell lines. The enzyme preparation (specific activity; 14.7 μmol min⁻¹ mg⁻¹ as assayed using phosphatidylethanolamine from *Escherichia coli*) at 40 μg ml⁻¹ did not induce the ability to form rosettes on lymphoid cell lines. In contrast, incubation of this preparation with human lymphocytes at 37 °C for 30 min showed a marked decrease in E-rosette formation, similar to that observed by Chapel with *C. adamanteus* PLA. Our experiments showed, however, that this treatment simultaneously degrades almost all the receptors for immunoglobulin Fc and complement C3 present on the non-treated lymphocytes and lymphoid cell lines, and also that it strips off the surface immunoglobulins⁴.

In contrast to the Crotalidae enzyme preparation, PLA highly purified from *Naja naja* venom induced the ability to form rosettes, but had no effect on the immunological receptors mentioned above. When the cells treated with PLA, even at concentrations greater than 640 μg ml⁻¹, were incubated at 37 °C in medium containing a foetal calf serum, they grew exponentially at the same rate as did non-treated cells, and the ability to form rosettes disappeared from the cell with time⁵. During these experimental periods no markers other than E receptors changed.

Thus, we agree with Chapel's arguments that among the effects of PLAs from different sources may exist a striking difference which has practical importance for the further study of E receptors. We should emphasise, however, that the PLAs of *Naja naja* and bee venom, though the latter exhibited only a weak activity on the human lymphoid cells, were specifically active in inducing the ability to form rosettes of the cells.

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reviews

Chemical kaleidoscope

Peter Farago

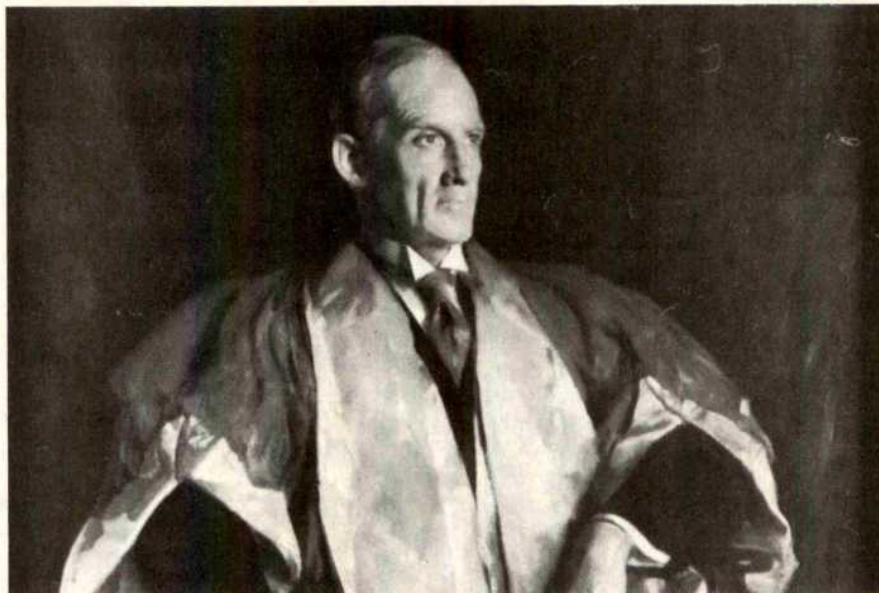
Memoirs of a Minor Prophet: 70 Years of Organic Chemistry. By Sir Robert Robinson. Pp. viii+252. (Elsevier: Amsterdam, Oxford and New York, 1976.) Dfl.49.50; \$18.95.

I LAST met Sir Robert Robinson a few months before his death in 1975. He looked very old. His sight had become weak and he moved with difficulty. But, sitting behind the big polished desk in his office high above the Thames, he suddenly became animated as he described the research he was still directing. His judgements on people and events showed none of the hazy mellowness to which an 88-year-old is surely entitled.

Robinson has been described by Lord Todd as "one of the few men of science to illumine by their own genius a wide variety of areas in their chosen subject" (*Chem. Brit.*, 11, 296, 1975). Within the international community of chemists, there were, and are, many deeply attracted by Robinson both as a man and as a scientist; yet one must record different reactions also. Nobody working in organic chemistry during the period 1915 to the mid-1950s could remain uninfluenced.

His book is a series of episodic recollections of people, places and scientific work, from early childhood to appointment to the Waynflete Chair of Chemistry at Oxford in 1930. One can compare it to the autobiographical writings of another great chemist, *Aus meinem Leben: von Arbeit, Musse und Freunden*, by Richard Willstätter (edit. by Arthur Stoll, Verlag Chemie, Weinheim, 1949), a tragic and human document about the fate of an outstanding scientist during the cataclysm of 20th century Europe. *Memoirs of a Minor Prophet* does not pretend to those heights because, one suspects, what really interested its author were ideas and things, rather than the human condition. As has been said about one of his great protagonists, he talked to molecules. If he suffered anguish, if he lay awake at night wrestling with the agonies of personal decisions, his book does not tell us about it. Emotions seem to have been fully engaged only with the ideas, substances and practitioners of science.

The autobiography presents a kaleidoscope of chemical ideas and personal views in the context of the first 30



years of this century, when organic chemistry was going through one of its most creative periods. Starting from a comfortable West Country middle-class background, the future Sir Robert passed through a Moravian Church school to Manchester University where H. B. Dixon, a pupil of Vernon Harcourt, was senior professor. Robinson entered the laboratories of W. H. Perkin, Jr, as a research student and started his academic career in the newly established Chair of Pure and Applied Organic Chemistry at Sydney in 1912. In the next 16 years he held five other appointments. Returning to a Chair in Liverpool in 1915, he spent a short, uncomfortable interlude with British Dyes Ltd in Huddersfield, followed by a Chair in St Andrews in 1921.

He returned to Manchester as Professor of Organic Chemistry in 1922 and transferred to University College, London, in 1928. The present book ends on the eve of his departure for Oxford. At each station of his progress there are descriptions of some colleagues and of research work in progress. The point of view is explicitly personal, and not all his contemporaries or those who followed would be expected to agree with it.

This was a most important period not only for science but also for scientists, whose contributions to the social fabric were being generally recognised; but there seem to have been few bridges between the worlds of university, in-

dustry and government the author describes. Of Robinson's contributions both as a theoretician, a practical chemist, and later as an industrial consultant, there can be no doubt. Whatever controversies surrounded some of the theoretical work, whole new fields of organic chemistry were explored by Robinson and his collaborators, laying the groundwork for spectacular insights not only in chemistry but also in many of the life sciences.

There are indications of all these strands in *Memoirs*, but the themes find their context only in terms of the author's personal impressions. Organic chemists will find vignettes of people, occasions and ideas which show the origin of a number of current developments in the science. They might usefully compare their present pre-occupations with those of Robinson's generation, observing carefully not only those ideas treated in the book but those which are absent. For those who are not chemists, the need for highly specialised knowledge of the subject will create a formidable deterrent to understanding. But even for them *Memoirs* does dispose of, yet again, the mythical unemotional scientist, the desiccated calculating engine.

Beautifully produced and not unreasonably priced, the book is utterly disgraced by the lack of subject and topic indices. □

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Diversity of the ecosystem

Ecological Diversity. By E. C. Pielou. (Wiley-International: New York and London, February 1976.) \$16; £8.

It is perhaps idle to speculate on what future generations of ecologists will, with their hindsight, deem to be the important developments of the mid-1970s. One area of ecology, however, that almost certainly will not go unheeded is the complexity-stability problem: not that this is a problem which originated in the mid 1970s but rather that there has been a plethora of approaches to the problem during the past four or five years. The arguments will remain, but one aspect of the problem that has occupied several ecologists is the measurement of the diversity of the ecosystem. Diversity, or complexity, can be measured virtually instantaneously whereas stability, the other side of the problem, can only be measured by observation over a long period of time.

Professor Pielou's book is thus a timely account of the one side of this ecological problem. The introductory chapter includes a brief review of the indices for measuring diversity and

equitability, and stresses particularly the different conceptual treatment of fully censured, as opposed to sampled, communities. The next three chapters provide a useful survey of the models and distributions that have been proposed to account for the number of individuals within the species of a community, and of testing hypotheses about species abundance. Again a distinction is made, being between the type of model based on ecological considerations (for example, the broken stick and niche pre-emption models) and the statistical model (for example, the negative binomial distribution).

The book is concluded with a series of four chapters which could be described as applications and 'odds and ends'. One of these chapters deals with spatial mosaics, with several pages devoted to the use of Markov-type matrices for analysing sequences along a transect, but in an example the categories used are 'bare ground', 'herbs', 'grasses and sedges' and 'tree seedlings', which seem to be a far cry from the precision of the first four chapters. Two other chapters contain a consideration of eight "determinants of diversity"—such factors as the diversity on small islands or the co-existence of potentially competing species. It seems here as if Professor

Pielou is trying to discuss a few areas in which she has personally worked, and thus the coverage of the subject is far from complete (for example, ecological succession is probably one of the more common factors affecting diversity, but this only receives a passing reference in a section dealing with the geological timescale).

Ecologists should take note of this book, particularly as a more rigorous assessment of diversity is likely to be required in approaching the complexity-stability problem. I very much doubt if they will. The extremely mathematical style will put many readers off, which is more the pity since some of the ideas of the last four chapters could usefully be more widely applied and developed. The somewhat obscure style of writing—for example, the third property of diversity indices in section 1.2 which is poorly described—is very frustrating for the reader. But the subject matter shows that there is still a lot of research to do in this field, and I hope the book will point out current deficits in our knowledge.

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Xenopus as a model system

From Egg to Adolescent: Xenopus—A Model for Development. By Louie Hamilton. Pp. xi+78. (English Universities Press: London, March 1976.) £3.45.

AMPHIBIANS have long been one of the most popular organisms in embryological laboratories, and in recent years *Xenopus laevis* has become the most widely used amphibian species. Its first important application was in nuclear transplantation, for which it was remarkably, perhaps fortuitously, well adapted. These experiments provided us with the strongest available evidence that all the cells of an organism contain identical genes, and in turn that development involves the continuous regulation of genetic activity. Coming on to the scene as late as it did, it is natural that *Xenopus* has made its main contributions to molecular biology, and to the infant science of molecular embryology. Moreover, certain biologists moved from developmental studies to the chemical dissection of gene structure, without changing their chosen organism. The result is that more is now known about the structure of some genes of *Xenopus* than of any other higher organism.

All of this must colour our expectations in reading Dr Hamilton's book. The reader will find a valuable summary of the main morphological events in the development of *Xenopus*, with very brief references to attempts at analysing these processes. There are many good pictures, especially electron micrographs, but the text suffers in general from a lack of diagrams that would help the reader to follow the complex morphological processes by which the vertebrate body is formed. It is, for example, very difficult to follow the formation of the nervous system in the absence of diagrams.

Nevertheless, the elementary student will find the description of development useful, and from time to time he will find his interest titillated by references to experimental embryology. Sadly, the book will not satisfy any interest it arouses because so few references are given. Surely the corpus of work on *Xenopus* justifies more than thirty-seven references. The molecular aspects of development are combined with nuclear transplantation in a chapter only four pages long, and with only two references. One feels that *Xenopus* deserves a little more! H. R. Woodland

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Proteases and biological control

Proteases and Biological Control. (Cold Spring Harbor Conference on Cell Proliferation, Vol. 2.) Edited by E. Reich, D. B. Rifkin and E. Shaw. Pp. x+1021. (Cold Spring Harbor Laboratory: Cold Spring Harbor, New York, 1975.)

THE very many different ways in which proteases contribute to biological control systems is the strongest single impression given by this conference report. Thus, although proteolytic enzymes have been studied for more than three-quarters of a century, it is only recently that it has become apparent how many different control mechanisms depend on these enzymes.

The work of Bayliss and Starling (1902) on the conversion of trypsinogen to trypsin by enterokinase is mentioned as an early example (K. A. Walsh). The first section on structure-function relationships also contains a valuable contribution from H. Neurath on limited proteolysis and zymogen activation. In the report of a meeting devoted to proteases and biological control, it is hardly surprising to find sections on coagulation, complement and kinins, and fibrinolysis. All, however, are excellent and have given the individual contributors the opportunity both to present recent results and to review their special fields.

The more fundamental aspects of protease action are made more comprehensible by a contribution on the specificity of proteinases toward protein substrates (J. S. Fruton). Also contributing towards an understanding of physiological control systems is the section on proteinase inhibitors in which the roles of both α_1 antitrypsin and α_2 macroglobulin are discussed. A number of rather disparate contributions appear in a section entitled Cellular Aspects of Proteinase Action. In one mainly devoted to protein turnover in animal cells, R. T. Schimke raises some fundamental question—such as the reason why proteins undergo continuous degradation *in vivo*. As he points out, a partial answer may be the possibility that about 15% of all proteins contain substitution errors incurred during synthesis. Another important reason for general turnover is also considered, that in its absence, the degradation of particular proteins, needed as part of many physiological changes, would require a set of specific proteinases as numerous as the proteins to be degraded.

A final section on tumour-associated proteinases contains several interesting contributions—for example on plasminogen activators, which are reported

to increase in concentration by a factor of 50 after neoplastic transformation of primary fibroblasts.

In view of the wide coverage, excellent quality of the individual contributions and rapid developments occurring in the field, the book deserves a warm welcome. **A. H. Gordon**

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Ornament of our times

Handbook of Psychopharmacology. Section 1: Neuropharmacology. Edited by Leslie L. Iversen, Susan D. Iversen and Solomon B. Snyder. Vol. 1: Biochemical Principles and Techniques in Neuropharmacology Pp. xiii+298. \$27. Vol. 2: Principles of Receptor Research. Pp. xii+288. \$27. Vol. 3: Biochemistry of Biogenic Amines. Pp. xii+486. \$37. Vol. 4: Amino Acid Neurotransmitters. Pp. xi+317. \$30. Vol. 5: Synaptic Modulators. Pp. xii+381. \$35.40. Vol. 6: Biogenic Amine Receptors. Pp. xii+307. \$30. (Plenum: New York and London, 1975.)

AFTER listening at a Journal Club to a presentation of the latest contribution of Scandinavian psychiatric epidemiology, Sir Aubrey Lewis was once heard to remark that this was not the sort of work which one could criticise, but was an *opus magnum* simply to be admired. The audience was somewhat astonished, never previously having detected any faltering in their professor's critical appetite. After delivering this one line, however, Sir Aubrey proceeded unabashed in his usual sharp and telling style, and everyone was much re-assured.

This set of six books on basic neuropharmacology represents only the first of three runs which will together comprise the complete *Handbook of Psychopharmacology*. We are therefore at this point looking at only part of a very large design. But even at this early stage we must unequivocally welcome the production of an ornament of our times. Here is a work which bears witness to the excitement and distinction of a branch of scientific investigation which has burgeoned over recent years. There is nothing in these books which is plodding or pedestrian, and nothing of the anxious pedantry which is sometimes the stuff of the *Handbook* which is desperately determined to miss nothing. A galaxy of scientists from many countries have contributed. To attempt the usual re-

view summary of contents would inevitably mean such condensation as to be misleading. It is sufficient to say that every important basic scientific aspect of this subject is authoritatively dealt with, and the subject explored out to its frontiers.

So much in essence for Sir Aubrey's opening statement. Following his example, it would however, be churlish not to offer some critical comment on an enterprise so eminently worthy of serious scrutiny. The largest problem which the editors had to face was obviously that of how to order and sort all the various issues, so as to produce a work which served the likely purposes of many different readers. There can be no absolutely satisfactory way of meeting such a sorting problem—to confine the coherence of one set of themes to entire chapters, other themes may inevitably be scattered between volumes. As a sort of test run, it was therefore interesting to look up the entries for phenothiazines in the index of each of these six volumes—no doubt the drug will again be treated in a later psychopharmacology section. What transpires is that in Volume 1 there is discussion of immunochemical assay (which certainly stands by itself). In Volume 2 the drug gets a mention in the EEG Chapter (blocking of dopaminergic transmission), and in the discussion of structure-activity relationships. Influence on 5-hydroxytryptamine and bearing on neuropharmacological theories of schizophrenia comes in Volume 3, whereas in Volume 4 the effect of chlorpromazine on brain GABA levels, and glycine and glutamate uptake, is discussed; and again the blockage of dopamine receptors. In Volume 5, influence of phenothiazines on brain energetics is dealt with (touching again on 5-hydroxytryptamine and dopamine), and later the inhibition of response to norepinephrine. In Volume 6 aspects of the dopamine questions come up once more, and in discussing the structural modification of phenothiazines and related dopamine receptor-blocking, line illustrations have to be produced which hark back to Volume 2.

There might in the next edition be further room for editorial tidying up, but the design of the enterprise in terms of basic science first and clinical application only later, is probably right and inevitable. It is Sir Aubrey's opening statement which stands.

Griffith Edwards

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Cinnabar elixirs to insulin

Science and Civilisation in China. Vol. 5: Chemistry and Chemical Technology. Part 3: Spagyric Discovery and Invention—Historical Survey from Cinnabar Elixirs to Synthetic Insulin. By Joseph Needham. Pp. xxxv+481: plates 462–476. (Cambridge University: Cambridge and London, May 1976.) £16.

PART 3 of Volume 5 of *Science and Civilization in China* completes the survey of alchemy begun in Part 2 (for review, see *Nature*, **254**, 539, 1975). It is more difficult to read than the earlier part because it analyses in detail material which is quite unlike anything with which the Western historian of science is likely to be familiar. Before Needham and his collaborators have been able to get down to any kind of criticism they have needed to examine the material written in a language which differs from Western languages in its basic make-up. It would be an impertinence on the part of any reviewer who, like myself, does not know Chinese, to make any remark on this subject were it not for one important fact about the book itself. Needham goes to a great deal of trouble skilfully to expound these linguistic difficulties and so makes it clear how he set to work and how he solved his problems. In the course of explaining some of the most recent chemistry he has some striking things to say about the different ways Western terms have been adapted to Chinese. His exposition of the language problem becomes a contribution to the history of chemistry.

More than in Part 2 he is concerned with individuals and the extensive record of their work. To follow all of these one needs to know a great deal of chemistry. It will not be easy going for those who do not. As in previous volumes one finds light being shed on Western science. For example, in dealing with Ko Hung, a great alchemist, and his contemporaries, Needham says "How did they keep their heads in the midst of so much religious-magical enthusiasm? How did he manage to make so many true observations of chemical behaviour, and carry out so many interpretable experiments even though he himself could never interpret them? . . . Ko Hung had one eye on the magic, the sacrifices, the Taoist temple liturgies but he kept the other firmly fixed on the real changes and transformations which he observed at his bench and his furnaces". We could apply this analysis to much that is puzzling in Western practice not only in the obviously related field of Western



A woman alchemist, possibly Thai Hsüan Nü, compounding elixirs. Taken from the Lieh Hsien Chhüan Chuan (Complete Collection of the Biographies of the Immortals), Ming, about 1580 AD.

alchemy and chemistry, but much other science and proto-science.

The fate of alchemy in China is curiously parallel with its fate in the West. It declined through the Middle Ages and was made the subject of satire. There was even a kind of iatrochemistry as its successor. But instead of a continuous suppression of alchemy by a progressive modern chemistry, there came a gap. Western chemistry was introduced into China bit by bit, to be adapted and assimilated like many other foreign influences, so that in

China, it seems, modern chemistry is the heir of the West, not of the remarkable, indigenous chemistry with which the major part of this book is concerned.

The title suggests that Needham has told a continuous story, but in fact he says plainly that he is not dealing with modern chemistry; the detailed treatment stops at the end of the eighteenth century, with only a few pages on the modern sinisation of terminology, but any more than we are offered would be indigestible.

There is one thing to regret. Needham refers to early illustrations of chemical apparatus but they are not reproduced. Perhaps it was not possible. There is, however, always work to do and we shall look forward to seeing them another time. The general impression is of the predominance of elixir-seeking in Chinese alchemy, and its relationship to the Chinese attitude to length of life as a virtue as well as an advantage. Resemblance to Western alchemy often arises from the fact that the Chinese were dealing with the same chemical substances, so that what they could discover and what they could infer was bound to be the same in the West. But the differences lay in the civilisation itself. Thus, the common ground of science enables one the better to understand what was distinctive in the civilisation. This justifies, if nothing else does, the title of the whole enterprise.

Frank Greenaway

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General relativity for undergraduates

Gravitation and Spacetime. By Hans C. Ohanian. Pp. xiv+461. (Norton: New York, May 1976.) \$18.95.

OVER the past decade there has been an explosion of discoveries, both theoretical and observational, in the subjects of gravity and relativity. Yet curiously there are still very few well written, broadly based and concise introductory books on general relativity which cover these exciting new developments. Perhaps this reflects the tradition of regarding general relativity as primarily a postgraduate and research activity; but increasingly in recent years, universities and colleges are running general relativity courses for undergraduates. This book by Ohanian is ideally suited to such courses as a teaching textbook.

The flavour of the book which emerges, suggests that it is aimed primarily at the physics student. The mathemati-

cal development of the subject matter is competent, and quite adequate for physicists, but perhaps a little sparing for mathematics students primarily interested in the modern techniques of differential geometry and topology, the singularity theorems, and so on.

The book contains much interesting discussion of experimental techniques and results. The later chapters cover modern developments in black hole theory and cosmology. There is little in the way of special relativity.

Many people will find this book useful for reference. Information retrieval is easy, the presentation of the material clear, concise and accurate; students should have little difficulty in finding their way around the subject matter, and exercises are plentiful.

In short, a solid, direct and informative reference and teaching book to be recommended to all students of relativity.

Paul Davies

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Parvoviruses

The Parvoviruses. (Virology Monographs/Die Virusforschung in Einzeldarstellungen, Vol. 15.) By G. Siegl. Pp. iv+109. (Springer: Wien and New York, 1976.) DM56; \$3.86.

THIS excellent little monograph reviews information on known members of the genus *Parvovirus* in the Family Parvoviridae. The volume of data accumulated during the past decade on members of this genus is so great (over 300 major references), and is so complex, that Dr Siegl has wisely concentrated on this genus, leaving information on the genera *Adeno-associated virus* and *Densovirus* to subsequent reviewers.

The intense interest engendered in this newly discovered family of viruses derives from their unique structure, a single-stranded DNA molecule capable of coding for only 3–5 proteins being enclosed in a 20-nm protein capsid. The dynamics of replication of such a single-stranded DNA in eukaryotic cells has provided an exciting challenge, and offered an ideal experimental system to study biochemical processes which occur during replication of DNA viruses.

For each of the nine members of the genus, Dr Siegl traces the work which has led to the finding that these viruses require cellular helper effects to replicate, and in consequence multiply only in actively dividing tissues. Although only two of the nine members have as yet been shown of importance in natural disease, the results of fundamental research on the 'important' members have shown these viruses to have a unique pathogenesis, selectively attacking foetal, neonatal, intestinal and haemopoietic tissue. This finding has in turn stimulated applied virologists to search for disease syndromes to associate with other members of this genus.

Dr Siegl has presented his review in sections, dealing with each virus on a species basis. Although this approach has inevitably led to some duplication of general information, it provides for easy reference by the species specialist, and certainly minimises the confusion associated with some members of the genus. Such complex data are particularly evident for the section on hamster osteolytic parvoviruses, and for those viruses which have been found as contaminants of cell lines. The review given for the porcine parvovirus has been subsequently developed (see Joo and Johnson, *Porcine Parvovirus—A Review*, *Vet. Bull.*, **46**, 653; 1976), and this virus has now been shown to

be a significant cause of natural reproductive failure.

Although information on the significance in nature of the porcine parvovirus and the feline virus have now clearly been established and control measures undertaken, it is disappointing to the reviewer as an applied worker, to see the paucity of information available on the disease significance of the bovine and canine parvoviruses. It is hoped that the future may see as detailed and conscientious a study of the significance of these viruses, and of possible human parvoviruses, as has been presented by the fundamentalists for the rodent viruses.

Although not in any way wishing to detract from the value of the monograph, the reviewer would have welcomed wider discussion and speculation by such a respected authority as Dr Siegl, and a gentle rebuke is included for the publishers in allowing printing of this excellent work without any obvious attempt to correct spelling and grammar.

R. H. Johnson

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Prostaglandins

Prostaglandins: Chemical and Biochemical Aspects. Pp. xiii+252. £9.95. *Prostaglandins: Physiological, Pharmacological and Pathological Aspects.* Pp. x+367. £11.50. *Prostaglandins and Reproduction.* Pp. x+332. £11.50. (Advances in Prostaglandin Research.) Edited by S. M. M. Karim. (MTP: Lancaster, 1975–76.)

As Professor Karim points out in his introduction, it is now impossible to comprehensively cover the entire prostaglandin field in one volume; this latest contribution is accordingly divided into three parts. The complete set comprises more than 20 chapters written with varying degrees of clarity by almost 30 specialists.

Part I surveys current understanding of the role of prostaglandins in reproduction. There is a general discussion of the role of prostaglandins in the reproductive physiology of man (Karim and Hillier), subhuman primates (Kirton), laboratory (Labhsetwar) and some domestic animals (Flint and Hillier). There are also chapters on the use of prostaglandins to induce labour (Thiery and Amy) and to interrupt pregnancy (Karim and Amy). This volume concludes with an interesting chapter on the use of prostaglandins

and their analogues in animal husbandry (Cooper and Walpole).

Part II deals with the more basic chemical and biochemical aspects of prostaglandin research, beginning with a review by Schneider of chemical synthetic methods for prostaglandins. The methodological information in the chapter on prostaglandin analysis by Salmon and Karim is particularly welcome, as is a thoughtful contribution by Lands and Rome on inhibition of prostaglandin biosynthesis. Sanner and Eakins review prostaglandin antagonists, and in the final chapter Kuehl, Cirillo and Oien attempt to clarify the confused area of prostaglandin–cyclic nucleotide interactions.

Physiological, pharmacological and pathological aspects of prostaglandins are covered in Part III, with interesting chapters on the role of prostaglandins in the central nervous system (Cocconi and Pace-Asciak), autonomic neurotransmission (Hedqvist), ocular (Eakins), gastrointestinal (Bennett), renal and cardiovascular (McGiff and Malik) as well as respiratory (Smith) physiology and blood coagulation (Howie). There is a rather short discussion on prostaglandins in inflammation (Greaves) and a welcome review of prostaglandins in tumours (Karim and Rao); this volume concludes with a useful review of the pharmacology of prostaglandin analogues (Karim and Ganesan Adaikan).

In rapidly developing areas, it is always difficult for reviewers to incorporate the most recent developments. Since 1974, there has been accumulating evidence that in some tissues, a large proportion of prostaglandin endoperoxides are metabolised to non-prostaglandin end-products and that in some cells (for example, platelets) the major biological actions are almost certainly due to the endoperoxides rather than the 'primary prostaglandins' E and F. This important idea was neglected by several contributors. It is regrettable—but unavoidable—that details of the most recent endoperoxide rearrangement products, the thromboxanes, could not be included.

I found this series worthwhile. It contains an abundance of up-dated material and covers much ground never before reviewed. The volumes have been well produced and the standard of the printing and illustrations was generally good, although there were some badly drawn structural formulae in one chapter. These volumes will be a useful addition to any departmental library; the cost of the series puts it beyond the pocket of most individuals.

R. J. Flower

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Agronomy and nutrition of maize

Mineral Nutrition of Maize. By I. Arnon. Pp. 452. (International Potash Institute: Bern-Worblaufen, Switzerland, 1976.)

THIS excellent volume is really two books in one, covering both agronomy of maize and general plant nutrition and fertiliser use with maize as an example. Following an introductory chapter on the origins, economic importance and botanical characteristics of this crop come several chapters discussing, in some detail, methods for maize production. These include sections on cropping systems, rotations, tillage practice, planting, control of weeds, pests and diseases, irrigation and harvesting.

The author reviews the most recent developments in these—for example, minimum tillage and use of herbicides—and draws together information from many parts of the world in discussing their impact on maize growing. A brief chapter on the general nutritional requirements of maize leads to a treatment of the nutrient supplying power of soil and the dynamics of nutrient uptake that would not be out of place in a soil science textbook.

The author is to be congratulated on the clear way in which relatively new concepts of the plant-root system are presented. As the author points out in the foreword, "more fertilisers are probably used for maize than for any other annual crop" and with "its high yield potential, the maize plant has been subjected to more research than most other crop plants". The results of this research have been excellently compiled in chapters dealing with the effects of fertilisers on yields and crop quality, on the important matter of interactions between nutrient elements and on practical aspects of fertiliser use such as soil testing, choice of fertilisers and methods and timing of applications.

Particular attention is paid to the relationship between fertiliser use and the local environment, management practices and maize varieties. Although concentrating on maize, these chapters deal with basic principles of fertiliser use which are relevant to many other crops and situations, making the book valuable even to those not directly interested in maize itself.

The entire book is a fine blend of scientific principles, research findings and practical aspects of crop production that should make it a welcome addition to the bookshelves of anyone

whose interest is in agricultural development. It is well produced, with many graphs, diagrams and tables, and several photographs including some in colour. The only criticism is the lack of an index, or even a list of illustrations, but the overall planning of the chapters makes it relatively easy to find the information required.

Stuart G. McRae

Stuart McRae is a Lecturer in Soil Science at Wye College, University of London, UK.

Phosphorus chemistry

The Chemistry of Phosphorus: Environmental, Organic, Inorganic, Biochemical and Spectroscopic Aspects. By John Emsley and Dennis Hall. Pp. xi+563. (Harper and Row: London and New York, July 1976.) £22.50; \$39.50.

SINCE the appearance about fifteen years ago of the monumental two-volume treatise by Van Wazer on *Phosphorus and its Compounds*, there has been a veritable explosion of monographs and series devoted to various aspect of this important element. Sasse, Hudson, Mann, Corbridge, Allcock, Kosolapoff and Maier (seven edited volumes); *Topics in Phosphorus Chemistry*, and *Organophosphorus Chemistry* (Annual Specialists Reports by the Chemical Society) are just a few of these solely devoted to various aspects of this subject.

Phosphorus chemistry in all its branches—biochemical, organic, inorganic, pure and applied—has made very great strides. Even so, one must closely scrutinise the appearance of a new book, particularly in a field in which so many authors with an international reputation have already put pen to paper. This is particularly pertinent at a time, when one has annually the unpleasant task of deciding which journal subscription has to be cancelled and to choose the very few books (from a long list) the library can afford to purchase (most monographs have already priced themselves out of the market for the individual buyer). As a contributor to Kosolapoff and Maier, I am aware of the pitifully small number of volumes of the standard reference treatise in an important field, which have been sold.

Professor Denney, in the preface, mentions that this is the first book attempting to bring together the various aspects of phosphorus chemistry under one cover. This is indeed the case. But for whom is this book in-

tended? Even a devotee of the subject like myself would hesitate to recommend a book of over 500 pages to undergraduates; it is an important and fascinating facet of Chemistry, but so are many others! In any case they could not afford it. The specialist research workers would require considerably more detail than could possibly have been given in a book of this size. A multi-authored multi-volume treatise would be required for them, but at a price at which most libraries would balk.

I found the book easy to read, although traditionalists might object to colloquialisms, such as: "This suggests that what (POPh)₃ loses on the δ swings it gains on the π roundabouts". I have learned something from sections, where my knowledge is cursory; I was disappointed in those the contents of which I know well. Perhaps this is inevitable in a book of this size.

The authors do not state up to which date they have covered the literature; my own impression is up to 1972, with some references either to secondary sources or to some selected research teams beyond that (mainly 1973). This is probably not the authors' fault; there are inordinate delays in the publication processes for both books and journals.

Obviously in a book of this size one cannot please everybody. Westheimer's work on the hydrolysis of cyclic phosphorus esters is covered, but Wolf's work on spiroposphoranes is hardly touched. The section on nuclear magnetic resonance spectra is thin, and no mention is made of Harris' important work on second-order phenomena. The industrially vital phosphates are barely touched; and Corey's work on the Wittig reaction, and Niecke's and Scherer's work on the isolation (of the hitherto only postulated) three-coordinate five-valent phosphorus species receive no mention. Misinterpretations and misquotations of the literature will be found by the expert eye.

In spite of the above criticism, it would be churlish not to acknowledge the tremendous time and effort the authors have expended on their task, and when used with discretion this book will be useful to some readers.

Although I enjoyed reading the book, the lingering doubt remains: whether the treatment of a subject with this scope in mind, would not have been better served as a "Current Awareness" book at a quarter, or even less, of its length and price, which would then have suited undergraduates and the general scientific reader. R. A. Shaw

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nature

November 25, 1976

Half greater than whole

WITH the steady decline in the number of those who go into productive industry in Britain and the steady rise in the number of people worrying about the problem, before the end of the century we expect these numbers to be equal and sometime early in the twenty-first century to see the disappearance of the last industrial worker. The Commons Select Committee on Science and Technology is the latest to agonise over the trend (Commons paper 680, £1.15), and if anything may have helped to speed it on its way by publishing some bitter remarks from some of its witnesses. From Lord Bowden, formerly Principal of the University of Manchester Institute of Science and Technology, for example, about the hazards of industrial life, the rather unpleasant environment and the contempt of society at large for those who create the wealth which everyone wants to spend. Or from the Chairman of EMI, who declared that you wouldn't choose the industrial environment for security, money or professional satisfaction. Or even from Mr Varley, the Secretary of State for Industry, who said that 'to be a technologist or a manager within industry has been a pretty lousy job'.

The committee, in looking at university-industry relations, has bitten off more than it can reasonably chew. Some recommendations (see also page 311)—on the future of the National Research Development Corporation, on the moving of basic research out of governmental research laboratories, on the functioning of the Advisory Council on Applied Research and Development, on the establishment of a Minister of Science and Technology, and on the extension of the customer-contractor principle into the Science Research Council (SRC) with the Department of Industry (DoI) as customer for the applied research—seem too hastily put together and based on too scanty evidence to be taken seriously. For instance on this last issue the committee has failed to consider that SRC, unlike the other research councils, supports little applied research in its own institutions, and that the proposal would, therefore, let the DoI (already well equipped with laboratories) choose to support or kill research projects in university laboratories—not, surely, the committee's intention.

It would be unfortunate, however, if the veritable deluge of half-worked-out proposals in the second half of the report were allowed to overshadow a valuable analysis, in the first half, of the development of post-war governmental support for science and technology education. It discusses the failures of new institutions and institutions with new names to convince bright students in large numbers that industry offered exciting

prospects—a failure compounded by industry's own inability to project itself as a place where highly qualified scientists and technologists would reach the top. The report echoes a DoI assessment that 'the overall trend is a decline in the numbers and quality of qualified scientists and engineers and of supporting staff in key areas of manufacturing'.

Status and motivation seem to emerge as the villains of the piece. Young people do not see engineering as a worthy profession in which to exercise intellectual skills; as a result there is too little understanding of what industry in all its complexity is about, or willingness to participate wholeheartedly amongst those 'forced' into it. The Select Committee, however, having inveighed against 'the distressing habit of attempting to bestow status (on academic institutions) by changing names', proceeds to propose as one recommendation that Imperial College, UMIST, University of Strathclyde and some former Colleges of Advanced Technology should be labelled SISTERS (Special Institutions for Scientific and Technological Education and Research). This is a revival of a 1963 idea to give technology greater prominence. The Rector of Imperial College will no doubt be mildly surprised to find that the committee propose that applied science and engineering at Cambridge be also made into a SISTER 'to ensure the high status of SISTERS is recognised by the academic community'.

The SISTER idea, unfortunately, is as poorly worked out as most of the committee's other ideas. To issue these institutions with revised charters limiting their functions to training and research in engineering and applied sciences would be to block all the fertile, increasingly necessary and in Britain often poorly developed cross-linkages into pure science, mathematics and economics. A dull narrow-minded product you'd get.

And yet the committee do at times get near to the centre of the problem: from early on in education the claims of industry and engineering are nothing like clearly enough presented. How many schools ensure that their pupils see a cross-section of industrial environments? How many industries make a serious attempt to get through to undergraduates, even to the extent of using them in vacations? The claims of an academic life are constantly being impressed on youngsters by their educational environment. Very often their only sight of industry before graduating is of strike picket lines on television news. There are some cautious ventures going ahead in this direction of mutual understanding, but it is up to the DoI, the Department of Education and Science and the Confederation of British Industry to stimulate much more. □

Stocks and shares

Questions about fish stocks lie behind Europe's fishing limits debate. **D. H. Cushing** discusses the state of stocks in the North-East Atlantic

THE conservation of fish stocks in the North-East Atlantic (between Cape Farewell and Gibraltar) started with the Overfishing Convention of 1946 which prescribed minimum landing sizes for demersal fishes in the ports and minimum mesh size in the trawl cod ends at sea. The Convention was activated in the Permanent Commission in 1953 which itself was replaced by the North-East Atlantic Fisheries Commission (NEAFC) in 1964 in order to acquire the power to regulate herring stocks as well as demersal ones. Sixteen nations have adhered to it voluntarily to conserve fish stocks internationally. In the last three years the Third Law of the Sea Conference has established a quite different trend towards coastal state management.

The present object of conservation is the maximum sustained yield (MSY) in weight. Men dispute whether it is a proper objective and propose others of various forms. In the North-East Atlantic, however, most stocks are overexploited and fishing effort should be reduced to obtain the MSY. This means that fewer ships at less cost would gain a greater catch and the average catch of each vessel or catch per unit of effort would be considerably increased. In his book *The Fish Gate* (Faber, London; 1943), Michael Graham named this principle the Great Law of Fishing.

The major stocks taken by fishermen in the North-East Atlantic comprise the cod-like fishes (cod, haddock, whiting and saithe), the flatfish (plaice and sole) and the pelagic species (herring, sprat and mackerel). The stocks are estimated by natural regions such as the North Sea, Irish Sea, Barents Sea and so on, between which exchange of individuals is usually low. The state of the stocks by species is summarised in the accompanying table; they are given by region (such as the North Sea) and are classed in four groups according to the reduction in fishing effort needed to obtain the MSY.

There are three stocks at or approaching the MSY with present measures: North Sea plaice, North Sea whiting and the cod in the Barents Sea. A slight restraint is required in five stocks: West Scotland herring, Iceland saithe, Faroe haddock and West Scotland cod and haddock. Eight stocks would be brought to the MSY

with moderately reduced fishing effort: Faroe cod, saithe in the North Sea and in the North-East Arctic, and both flatfish species in the Irish Sea, Bristol Channel and the English Channel. Unfortunately, there are fourteen stocks in need of heavy reduction in fishing effort: cod in the North Sea, Irish Sea and Bristol Channel, haddock in the North-East Arctic, North Sea, Irish Sea and Bristol Channel, whiting in the Irish Sea and Bristol Channel, sole in the North Sea and Irish Sea and herring in the North Sea and Irish Sea. The state of sprat and mackerel stocks in the North Sea and of mackerel stocks in the Western Approaches is uncertain.

Three reasons

There are three reasons why, after twenty years, most fish stocks in the North-East Atlantic remain over-exploited. The first concerns a limit in mesh regulation. The impact of an increase in fishing effort on a single species can be lessened to some degree by enlarged meshes in the trawl cod ends, but in a mixed fishery the meshes are fitted to the smallest fish species and any increase in fishing effort on the larger animals cannot be accommodated in this way. In the North Sea, mesh regulation was designed mostly for soles and haddock but the benefit for larger species like cod and plaice was less. In the North Sea and elsewhere in the North Atlantic, minimum mesh sizes were agreed by the end of the



Quota problems go deep in Denmark ...

1950s. But during the 1960s a considerable increase in fishing effort occurred all throughout these regions. In the International Commission for North-West Atlantic Fisheries (ICNAF), quotas were introduced in 1970 and in NEAFC in 1974. From North-West Atlantic experience, quotas stabilise fishing and prevent further expansion.

The collapse of herring fisheries throughout the North-East Atlantic illustrates the second reason for over-exploitation. The Downs stock, exploited by the East Anglian fishery, failed in the late 1950s, followed by other stocks in the North Sea; when NEAFC took power to regulate herring fisheries it did not succeed in doing so. When the Norwegian fishery on the Atlanto-Scandian herring stock collapsed in 1967, no action was taken. A contributory but minor cause of failure was the mistaken belief that fishermen must take fewer herring than the whales,

State of the stocks in the North-East Atlantic: reduction in fishing needed to obtain MSY

	None	Slight	Moderate	Heavy
Cod	North-East Arctic ¹	W. Scotland	Faroe	North Sea Irish Sea Bristol Channel
Haddock		Faroe W. Scotland		North-East Arctic North Sea Irish Sea Bristol Channel
Whiting	North Sea			Irish Sea Bristol Channel
Saithe		Iceland	North-East Arctic North Sea	
Plaice	North Sea		Irish Sea Bristol Channel English Channel	
Sole			Bristol Channel English Channel	North Sea Irish Sea
Herring		W. Scotland		North Sea Irish Sea Celtic Sea

D. H. Cushing is Deputy Director of the Fisheries Laboratory, Lowestoft, UK.

¹At the present pattern of exploitation.

dogfish, cod and gannets that driftnetmen saw round their nets.

The major cause was the scientific dogma that fishing could never reduce the magnitude of the incoming year classes. Today we distinguish growth overfishing from recruitment overfishing. In growth overfishing the loss in numbers is greater than need be to give the maximum yield from the growth of individuals, and it is no accident that such individuals grow by an order of magnitude during their adult lives. In recruitment overfishing (often in herring-like fishes that grow little during their adult lives) the magnitude of the incoming year classes is reduced by fishing—an interference with breeding, as the fishermen used to say. The collapse of the herring stocks in the North-East Atlantic has been due to recruitment overfishing.

One of the difficulties was the high variability of recruitment; the dependence of recruitment on parent stock looked like a scatter diagram and sometimes the true relationships were masked. Indeed the belief that recruitment was independent of parent stock was mistakenly reinforced. The important conclusion, however, is that the overexploitation of the herring fisheries in the North-East Atlantic was less due to institutional failure than to the inability of scientists to understand the problems they faced. It remains true that the failure of NEAFC to take prompt and effective action was due in part to the difficulties of agreeing upon measures which would necessarily severely restrict the fisheries economies of some member states.

Considerable overexploitation

The development of industrial fisheries for fish meal in the North Sea has caused considerable overexploitation indirectly. During the 1960s herring were caught for fish meal on their spawning grounds and the trawlers were allowed to take 10% by-catch of those protected species which gathered to eat the herring spawn on the sea bed. Today more than two million tons of fish are taken from the North Sea for fish meal and the by-catches of protected species can be considerable. The sandeel fishery is 'clean' and little else but sandeels is caught. In the fishery for Norway pout (which are very small gadoids) in the northern North Sea, however, small haddock and whiting are caught in large numbers. In some years more than 100,000 tons of small haddock have been turned into fish meal, and in 1976 75,000 tons of small whiting were caught for the same purpose.

Norway pout are caught with small-meshed nets, legal under NEAFC rules, provided that the catch of under-sized protected species is less than 10%;

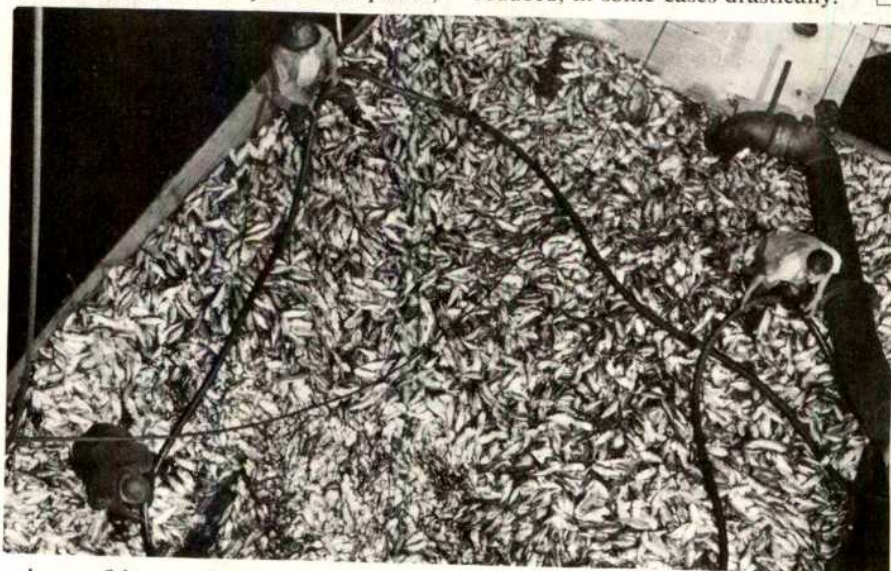
the total catch of Norway pout in 1975 exceeded 800,000 tons. The real difficulty is that fishermen exploiting adult gadoids for human consumption must not land fish below the minimum landing size and must discard them at sea, whereas fish meal fishermen can land large quantities of them; the large industrial fleets are seen everywhere in the North Sea and the traditional fishermen for human consumption see the small gadoids being taken which they themselves have to discard. Further, a rough calculation shows that 300,000 tons of Norway pout for fish meal are less valuable than 20,000 tons of haddock, had they been allowed to grow into the adult fishery.

After two decades of recruitment overfishing, the remaining herring stocks in the North Sea need drastic conservation measures. This need has at last been recognised by the industrial fishing nations who have agreed to a ban on all directed fishing for herring in the North Sea. But juvenile herring are caught in the industrial fishery for sprats; in 1975, about 600,000 tons of sprats were taken and the by-catch of small herring in the Danish catches was substantial. In other words, the industrial fishery for sprats not only prevents any chance of recovery of herring stocks, it also hastens the chance of extinction. A fair proportion of the world's catch of fish ends as fishmeal because of the high demand for it, and many industrial fisheries, like that for capelin in the Barents Sea or for anchoveta off Peru, exploit resources that cannot be used at all for human consumption. The problem peculiar to the North Sea is the conflict in use between fisheries for industrial purposes and those for protected species.

The three sources of overexploitation were recruitment overfishing, the conflict between fisheries for industrial species and those for protected species,

and the increase in fishing effort in ships, fishermen and gears. The herring and sole stocks in the North Sea are in danger from recruitment overfishing, but the problem has been recognised and steps are being taken in the scientific working groups to resolve it, even if the herring problem has not yet been solved in the Commission itself. The problems of the industrial fisheries are more difficult because of the large investment in ships and processing equipment in Denmark. Possible solutions include the diversion of such fleets on to stocks which would yield no by-catch of protected species, or to establish areas and seasons in the Norway pout and sprat fisheries when the by-catches of protected species are reduced to tolerable levels. The problem must be solved because it is hard to persuade the non-industrial fishermen to accept quotas or effort reduction when the industrial fleets are allowed to catch small gadoids and small herring.

The table shows that fishing effort should be extensively reduced throughout the North-East Atlantic. The introduction of quotas in 1975 has stabilised fishing effort; in ICNAF with a longer experience of quotas since 1970, an effort restraint was also introduced in 1976. The effort exerted on all the stocks on the right half of the table is much too great, which means that fleets in the North Atlantic will have to be reduced to some degree. There are few stocks left unexploited, but one is large, that of blue whiting off the west coast of the British Isles. Stocks are estimated at about 8–15 million tons and the potential catches could be large, but the exploitation has hardly started. Although some existing vessels could be used to catch the blue whiting, it remains the case that fishing on the great majority of other stocks in the North-East Atlantic must be reduced, in some cases drastically. □



... but overfishing problems go deeper

400 GeV . . . and beyond

Experiments begin soon on the 400-GeV Super Proton Synchrotron (SPS) at CERN, Geneva.

David Davies has been visiting the laboratory

SPS comes on line within budget and on schedule. After six years of awaiting the complicated multinational approval, and a further five of building an accelerator of radius 1.2 km which circles some 40 metres below ground, the physics starts in earnest in early December when the first high-energy particles are fed to a whole array of experiments. Major construction still continues, as the plans are for two completely separate experimental areas, only one of which is yet finished. But already questions are being asked: what do we build next?

CERN started life with a 600 MeV synchrocyclotron (SC) commissioned in 1957 and still in use. In 1959 a 28-GeV proton synchrotron (PS), fed successively by a 550-KeV electrostatic generator and a 50-MeV linear accelerator, was added. Although there is still an active physics programme on the PS, it also serves to feed the two other machines—the intersecting storage rings (ISR) and the SPS. ISR was completed in 1971, and comprises two rings of diameter 300 metres in which 28 GeV protons can travel for up to 40 hours in opposite directions crossing over and colliding at eight equally spaced positions—at six of which experiments are sited. The performance of the ISR in terms of storage current and luminosity is continually improving, and plans for its use well into the 1980s are being discussed at present (see page 314).

The advantage of machines in which beams travelling in opposite directions collide is that almost all the energy is available for particle production: SPEAR at Stanford and the projected PETRA at Hamburg are further examples, in which electrons and positrons are ranged against each other. In accelerators which bombard stationary targets, a large fraction of the energy goes in recoil of the target

particles. On the other hand, storage rings are restricted to a much smaller range of experiments—in other accelerators the primary beam is very often converted at a target to secondary beams of completely different particles.

The protons for the SPS are peeled off the PS in a continuous ribbon which eventually fills ten-elevenths of the SPS's seven-kilometre circumference. Once in the ring the 10^{13} protons encounter the magnetic field both of dipoles (which bend the protons in a horizontal plane) and quadrupoles (which keep the beam focused). There are 744 dipoles (peak field 1.4 Teslas) and 216 quadrupoles and the mean power consumption is 34 MW. The vacuum is 10^{-7} Torr. Acceleration of protons occurs at only one point on the ring, in a straight stretch of 40 metres where there are two radio-frequency cavities. Each journey through this section adds about 2.5 MeV to the protons' energy. After 150,000 circuits, which take 3.7 seconds, the protons are up to an energy of 400 GeV and ready to be ejected into either of the experimental areas, West or North. West Area is the first to be completed, North follows early in 1978.

The proton ribbon can either be extracted all in one turn or can be progressively peeled off over a period of up to a second. Bubble chamber experiments tend to need the short burst, electronic counter experiments the longer time. The beam can be used in the West Area in three ways:

- It can be directed on to an underground target at up to 40 GeV to produce pions and kaons which decay within 430 metres of their journey to the surface into muons and neutrinos. The muons are absorbed in steel and earth and the beam as it reaches the surface is solely of neutrinos which pass through the Big European Bubble Chamber (BEBC), two counter experi-

ments and the heavy-liquid bubble chamber Gargamelle.

- The protons can be directed to a second underground target to produce 75-GeV kaons or 110-GeV antiprotons which run into BEBC.

- The beam can be brought to the surface and split into three on entering the West Hall. Three targets are available to feed the twelve separate experiments set up in the West Hall with secondary beams of up to 150 GeV. The dimensions of the building—which existed prior to the SPS—preclude using higher energies; the North Area will be custom built for 400 GeV.

Why, it may be asked, has CERN built a 400-GeV proton accelerator when the Fermilab at Batavia, Illinois, installed a comparable machine more than four years ago—is it not needless duplication? The answer is complex and depends as much on subjective opinion as objective fact. But the list of SPS experiments approved at CERN gives some idea of the differences. Most striking are the number of neutrino experiments—13 out of 28. These take advantage of both the extremely high event rate anticipated from the neutrino beam-line and the excellent bubble chamber facilities. A second unique feature of CERN is the hyperon beam-line, yielding Ξ and Σ particles; an experiment on the decay characteristics of these particles will be watched with interest. A third will be the muon facility in the North Area.

It is also a mark of CERN's distinctive character that although roughly half of all experiments planned on the SPS are devoted to the "new physics"—hunts for J/ψ , intermediate vector bosons (maybe!), heavy leptons and charm—the other half tend to be "spectroscopic" experiments in the realms of "normal physics", painstakingly filling in gaps in knowledge without spectacular expectations.

Those who have worked in both Fermilab and CERN comment on the difference in style—Fermilab has a lot of flair, is able to latch on very quickly to new ideas, is vulnerable to failures (the magnets break down with

What SPS costs . . .

Item	Swiss Francs ¹ (millions)
Total CERN expenditure in 1975	647.9
of which SPS construction	237.9
SPS contract expenditure to October 1975: on	
site buildings and equipment	214.7
normal machinery components	194.6
special machinery components	89.7
Total	499.0
of which British contractors received	75.8
Projected total cost of building SPS (1970 prices)	1150

¹SFr 2.4 = \$1 SFr 4.0 = £1

. . . and who pays

Country	% in 1976	% in 1971
Austria	2.22	1.96
Belgium	4.02	3.77
Denmark	2.29	2.26
France	21.49	19.90
West Germany	25.40	23.27
Italy	13.34	12.89
Netherlands	5.30	4.43
Norway	1.60	1.52
Sweden	4.55	4.59
Switzerland	3.38	3.20
UK	16.41	21.61

Contributions are based on GNP

depressing regularity); CERN is more bureaucratic in its planning of experiments, is less prone to serendipity and yet runs so consistently well (SPS is expected to be extremely reliable) that much excellent definitive work can be done. As one physicist put it "at Fermilab you practically bring your experiments in cardboard boxes and stick them under tarpaulins; here look at the huge echoing halls and the gold-plated equipment!"

One of the problems that CERN is facing is the growing reliance of the high-energy physics community on one central facility as national facilities are tapered off. An exception is in Germany, where many reckon PETRA will produce some very exciting physics, and again will undoubtedly attract an international clientele; but otherwise there is nowhere else to go. Maybe the average number working on an experiment, which is 6 on the SC, 12 on the PS, 18 on the ISR and 25 on the SPS, reflects in part an increased complexity of experiment. But some believe that there are too many working on the SPS and that the quality of experience both for graduate students and research worker suffers accordingly. Another concern, raised first by Professor Jentschke last year when he was director of CERN's Laboratory I, is that very few physicists from CERN's smaller member states are finding their way into the teams for the SPS.

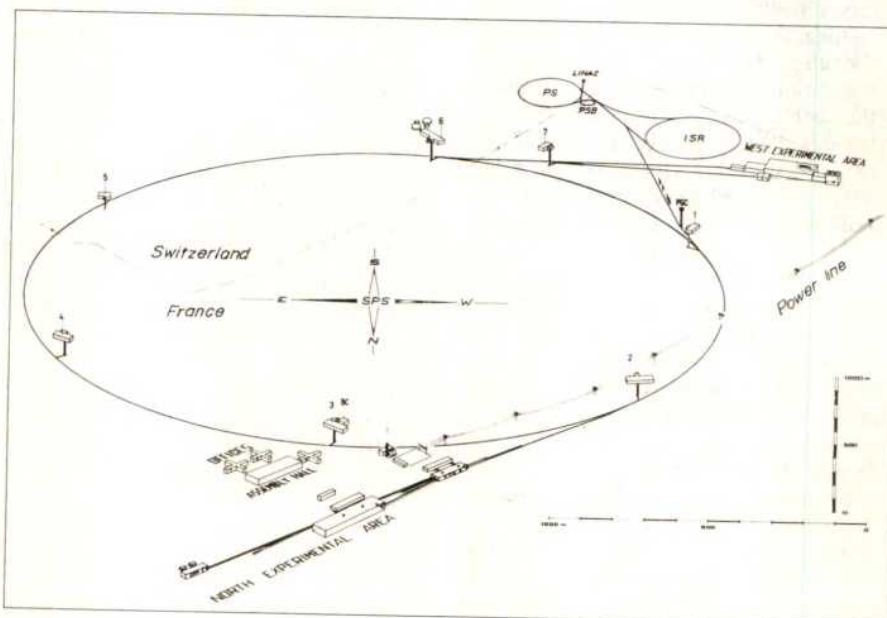
There is, on the other hand, some feeling around that CERN staff get a better deal than those who simply visit. The tax-free salaries are generous, to say the least, even allowing for the high cost of living in Geneva. And the cost of CERN's on-site services means that many (British, in particular) bring in their own technical staff.

Not surprisingly, there is a little apprehension at the moment at what financial adjustments the United Kingdom may try and negotiate at CERN's December Council. Dr J. B. Adams, the Director-General, is phlegmatic as always—"last year the Germans had some difficulties, the year before it was the French; these things come and go". But the real question is not a short term one. It is whether over the next five or ten years the nations contributing not only to CERN but also to other major accelerators are going to sustain their support for high-energy physics.

Within CERN itself there are already ideas circulating for new machines. The tunnelling for the SPS cost a relatively small fraction of the total bill, and some would like to see the SPS ultimately acquire an intersecting ring for proton/proton or even proton/antiproton studies. Such discussions,



Looking towards West Area from SPS



SPS plan, showing link with PS and experimental areas

however, are often coloured by a feeling that since the weak and electromagnetic interactions seem to be more fruitful sources of new states than strong interactions, a CERN venture into an electron machine might be a good next step. The European Committee for Future Accelerators has had a working party look at the physics potential of a 100-100 GeV electron-positron storage ring.

Fermilab, meanwhile, is exploring the possibilities of superconducting magnets to push its peak energy up to 1,000 GeV. The intellectual drive to get up to this level is that the postulated carrier of the weak force, the intermediate boson, probably has a mass so high (at least 37 GeV) that 1,000 GeV protons would be needed to unearth it. The prize from this could be unification of theories of weak and electromagnetic forces. CERN initially planned superconducting magnets in the SPS but eventually dropped back

to conventional ones because the technology was not far enough advanced.

At the same time Soviet physicists are looking to a 2,000 GeV proton synchrotron at the Institute of High Energy Physics, Serpukhov, as the next step from their 76-GeV synchrotron. Again, superconducting magnets are envisaged, to deliver the fields of up to 5 Teslas. Plans also include the simultaneous construction of a 20-GeV electron synchrotron with the capacity for proton-electron collisions.

It seems that plans for the next generation of machines are too far advanced in the United States and the Soviet Union for there to be any possibility of global collaboration. Attention has therefore turned to the idea of the next accelerator-but-one being a truly world machine, if only because a multi-billion-dollar price label is bound to be attached to it. A proton synchrotron at more than 10,000 GeV is a possible. But this is up to the politicians. □

USA

Where lies the test?

The Food and Drug Administration last week issued a set of regulations designed to help combat a growing scandal over drug testing in the United States. Colin Norman reports from Washington

A RELATIVELY unremarkable scientific paper, published early in 1972, has touched off a major scandal over the testing and regulation of drugs in the United States. It has led to allegations that some drug companies have conducted sloppy animal tests, faked results and presented misleading toxicity reports to the federal government—allegations which call into question the integrity of key parts of the drug regulation process and cast doubt on the safety of some products.

The allegations are so serious that the Food and Drug Administration (FDA) has already investigated the testing procedures and test reports of a few companies, and it is about to launch an unprecedented inquiry into the work of dozens of others. On the basis of what it has turned up so far, FDA last week issued a bulky set of regulations which essentially instruct the drug industry how to conduct animal tests and warn that drugs could be pulled off the market if it is subsequently discovered that misleading reports on them have been filed with FDA. The objective of the proposed new regulations, an FDA official said last week, is "to make sure that drug companies compete with each other on the quality of their professional work rather than on their level of cost-cutting".

The concern over the quality of drug testing was triggered by a study published in the *Journal of the National Cancer Institute* by Mario Rustia and Philippe Shubik of the Eppley Research Institute. The study indicated that a commonly prescribed drug, known as Flagyl, increases the incidence of pulmonary and lymphoid tumours in mice. The paper raised eyebrows in FDA because the agency had in its files reports of two studies submitted by the drug's manufacturer, G. D. Searle and Co., which claimed to show that Flagyl is not carcinogenic in rats.

A thorough check of Searle's study was then conducted by Adrian Gross, an FDA scientist. Gross concluded that Searle's study did indeed show evidence of carcinogenicity, but the company's report didn't reflect all the data. FDA officials took the matter up

with Searle, and two years later the company re-submitted its report. This time, Gross said in an interview last week, the summary agreed with the data, which would have been fine except that it was the data, not the summary, which had been altered. That finding prompted FDA to conduct a full-scale investigation into Searle's testing and reporting on several products. It was probably the most thorough federal investigation ever conducted of a drug company's scientific activities and it turned up some very disquieting findings.

The official report of the FDA investigation is among the most outspokenly critical federal documents that one could read. It states:

We have uncovered serious deficiencies in Searle's operations and practices which undermine the basis for reliance on Searle's integrity in conducting high quality animal research to accurately determine or characterise the toxic potential of its products . . . Searle has not submitted all the facts of experiments to FDA, retaining unto itself the unpermitted option of filtering, interpreting, and not submitting information which we would consider material to the safety evaluation of the product. Some of our findings suggest an attitude of disregard for FDA's mission of protection of the public health by selectively reporting the results of studies in a manner which allays the concerns of and questions of an FDA reviewer.

The task force which conducted the investigation recommended that the matter be turned over to the Justice Department, and that a grand jury be called to look into possible violation of the law. An FDA spokesman said last week that no grand jury investigation has yet been started, however. It should be noted that Searle's chief executive officer, Daniel Searle, acknowledged in Congressional testimony earlier this year that some "human errors" had occurred but he maintained that the FDA inquiry had found no evidence of fraud or misrepresentation by Searle personnel.

Whatever the outcome of the investigation, the Searle episode raises some very disturbing questions. FDA regulation of drugs and food additives is based largely on safety data submitted by the manufacturer of proposed new products. Thus, if manufacturers' test reports are unreliable, for any reason, much of the basis for FDA's regulatory actions could be open to doubt. And animal tests, it should be noted, are particularly important since they provide the chief means of identifying whether or not new products may have potential long-

term problems, such as carcinogenicity, teratogenicity, and mutagenicity—problems which don't show up in clinical trials on humans.

Because of the serious implications if FDA is regulating drugs and food additives on the basis of unreliable data, Congress earlier this year added \$16.4 million to the agency's budget for an investigation to determine whether the problems uncovered at Searle are widespread in the industry. Senator Edward Kennedy, who was instrumental in securing the extra funds and in bringing the matter to public attention, noted last January that "if data are proven false and misleading, then the regulatory decisions may be tragically wrong".

FDA's investigation is about to get under way in earnest. During the next three months, inspectors from the agency will look into testing procedures and facilities of about 40 drug companies and independent testing laboratories. But FDA has already gathered alarming evidence that the problems are not limited to Searle.

Last week, the agency proposed a set of so-called Good Laboratory Practice (GLP) regulations which spell out standards which FDA will require for animal tests of drugs and food additives. In a preamble to the proposed regulations, FDA Commissioner Alexander M. Schmidt states that a number of recent investigations conducted by FDA in cases where the agency had reason to be suspicious of test reports "have identified significant problems in the manner in which nonclinical laboratory studies are being performed". The deficiencies, Schmidt said, were uncovered during inspections of "major pharmaceutical firms, inspections of several private contract testing facilities, and internal reviews of toxicity studies of color additives conducted by FDA".

The problems included the following:

- "Pathology reports submitted to the agency were inconsistent with the original autopsy records".
- "Microscopic tissue slides were conducted by more than one pathologist, each of whom came to different conclusions, yet only the conclusions favourable to the drug were submitted to the agency".
- No records were kept for any individual animal in one long-term feeding study, "despite the fact that the records were required for proper analysis of the study and were represented to the agency to exist".
- "Animals were recorded as normal for a variety of factors, including

awareness, appearance, appetite, and thirst, when in fact the animals were dead".

- "Drugs were administered to animals in a manner which made it impossible to determine how much, if any, of the required dosage was actually ingested by the animal".

- In one toxicity study, "gross changes of tissue began to appear, yet management was not made aware of these alarming changes for approximately 4 to 8 months.

- In a study done by an independent testing laboratory, FDA was told that animal tissue samples had been examined when a review of laboratory records indicates that the tissue samples had never even been collected.

Such alarming, and possible fraudu-

lent, incidents are supposed to be stopped, or at least made more difficult, by FDA's proposed GLP regulations. But the regulations would in fact not be unduly burdensome. They set standards for the training of laboratory personnel, the quality of facilities and equipment, the handling of test animals, and the keeping and reporting of test data. They also specify that a Quality Assurance Unit must be established in each facility to ensure that the GLP regulations are followed. In short, the proposed standards are no more strict than those which should already be in force in any self-respecting facility.

Perhaps the most important aspect of the proposed regulations is that they spell out a number of sanctions which FDA could invoke if violations of the GLP standards are encountered. The ultimate sanction would be to

remove a product from the market if FDA had approved it on the basis of test reports subsequently found to be false or misleading. Another proposed sanction would be to disqualify a test facility as a source of information in support of a future marketing permit. Such action would be tantamount to shutting down a contract laboratory which makes its living from conducting toxicity tests for the drug industry. Finally, FDA points out that deliberate faking or misleading reporting of test results could be subject to criminal prosecution.

The proposed GLP regulations are open for comment for 120 days and FDA plans to conduct public hearings on them early next year. By that time, the results of the inspection effort should be available to provide an indication of the scope of the problem. □

SEVESO

The problems deepen

Italian authorities are now pondering how to clean up an area near Seveso in northern Italy, following an accident last July which released large quantities of dioxin into the environment. Some important new information bearing on the problem has surfaced in the United States. Colin Norman reports

DIOXIN in the soil of the most heavily contaminated area near Seveso can be expected to take up to 14 years to break down to levels at which the compound can no longer be detected. But there is unlikely to be much, if any, movement of dioxin in the soil, and the contamination will probably be confined to the upper 12 inches for a long time.

Those conclusions have emerged from an analysis of previously undisclosed studies conducted by the US Air Force, and from information gathered as a result of a bizarre dioxin contamination incident in Missouri in the early 1970s. The studies were ferreted out and analysed by Barry Commoner and Robert E. Scott, researchers at the Center for the Biology of Natural Systems at Washington University. Scott and Commoner have sent their conclusions to authorities investigating the Seveso disaster.

The Air Force studies, which were released to Commoner on October 28, involved soil tests with military herbicides contaminated with dioxin, left over from the Vietnam war. They involved heavy spraying of plots of land in Utah, Kansas and Florida, and subsequent monitoring of dioxin levels in

the soil. Commoner and Scott conclude from the studies that the likely half-life for breakdown of dioxin in the soil at Seveso is about 250 days, a rate which suggests that it would take about 14 years for contamination to disappear from the most heavily affected area (Zone A) and about seven years to degrade to undetectable levels (about 10 parts per trillion) in the surrounding zone (Zone B).

But, in spite of an annual rainfall of about 60 inches in Florida, the Air Force study indicated that dioxin in the test plot there did not migrate in the soil. That finding is particularly important because there has been recent heavy rainfall in Seveso which has led to worries that the dioxin may have been washed through the soil into the groundwater. "It may be expected that the TCDD present in the upper layers of the soil at Seveso will probably not migrate downward significantly, in spite of the heavy rainfall", Commoner and Scott conclude. Those observations lead them to note that if the soil at Seveso is left in place, contamination is unlikely to spread, but alternatively if the upper layers of soil are stripped off and replaced with fresh soil, any dioxin left in the lower layers would not be expected to migrate to the surface.

The Air Force study has also yielded information on the effects of low levels of dioxin on wildlife. Samples of wildlife around the Florida test plot indicated significant bioaccumulation of the contaminant, and there was also a marked drop in the fertility of beech mice in the area. The observations lead

Commoner and Scott to conclude that wildlife at Seveso will be affected unless soil concentrations can be reduced to about 500 parts per trillion, compared with the average concentration of about 10 parts per million in Zone A. Such a reduction, they note, "could be most rapidly achieved by removing the upper layer of soil and replacing it with uncontaminated soil".

The other information analysed by Commoner and Scott was derived from observations following an incident in which waste oil heavily contaminated with dioxin was sprayed on the ground in four places in Missouri in May and June, 1971. The dioxin contamination was unknown at the time.

One application involved spraying the ground in a horse arena shortly before a show to keep down dust. Hundreds of birds and several horses died as a result of exposure to dioxin and three months later a young girl who had been playing in the arena was admitted to hospital with symptoms ranging from nosebleeds and headaches to severe disuria and hematuria. In October, four months after the spraying, between six and eight inches of topsoil was removed from the area and replaced with fresh soil, but deaths of horses, cats and other wildlife persisted. In April, 1974, four years after the accident, a further six inches of soil was removed, whereupon the toxic effects disappeared.

The original contamination in the arena was reckoned to be about 30 parts per million, a level similar to that in Zone A, and thus the observations compiled by Commoner and Scott are directly relevant to the Seveso investigation.

Commoner and Scott conducted

their study under the auspices of the Scientists Institute for Public Information.

● **Alastair Hay adds:** At the time of the Seveso explosion, 150 women resident in the area were in the first trimester of pregnancy. Most of the women applied for a therapeutic abortion when informed of the teratogenic properties of dioxin. Following examination by health officials of 730 pregnant women formerly resident in the contaminated area, only 29 have had the operation. The fetuses removed from these women are currently being examined for any signs of abnormality.

Professor Francesco Cefis of the *Istituto Clinico di Perfezionamento* in Milan, and Professor Alfred Gropp, Head of the Pathology Department of Lübeck Medical School in Germany, are in charge of the investigations. Neither would comment last week on the results of their observations so far. Only five of the 29 fetuses have been examined and Professor Cefis estimated that it would take another two months to complete the study. By then women who were in the earlier stages of pregnancy in July will be six or seven months pregnant—too late for an abortion to be performed.

The rate of spontaneous abortions in the Seveso area is reported to have increased to twice the Italian national average since July. The rate of miscarriage was reported to have increased similarly in Vietnam following spraying with the herbicide 2,4,5-T. Both the trichlorophenol discharged at Seveso and the 2,4,5-T used in Vietnam contained dioxin. The Vietnamese authorities maintain that dioxin was responsible for the miscarriages

Towards anticipating disaster

In Britain last week the Insurance Technical Bureau (ITB), which leading insurance groups established in 1972 as a non-profit making organisation with "technical expertise in the field of industrial loss prevention", demonstrated 'Anticipator', a new system designed to alert managers of chemical plants to potential hazards in their site installations.

One of the recommendations made by the 1974 Court of Inquiry into the Flixborough disaster was that some form of recorder should be made available to monitor operating conditions in industrial installations. This would perform a function similar to that of the 'black box' flight recorder in aircraft. 'Anticipator' is a sophisticated monitoring system with its own computer which will register information about pressure, temperature, flow rates,

gas leaks, vibrations and other factors relevant to the safe operation of a chemical plant. It can be pre-programmed to record only data which fall outside normal working parameters, making interpretation a relatively simple process.

The designers argue that incidents usually "cast their shadows before them", and feel certain that Anticipator would have prevented accidents such as those at Flixborough and Seveso. With the primary research and development work now completed at a total cost of £50,000, the ITB propose to find a commercial concern to develop the system for marketing. The trouble is, the biggest firms do their own monitoring, and the smaller ones may not be able to afford the new device.

Alastair Hay

which they recorded.

Meanwhile, the scientific commission responsible for formulating a policy to decontaminate the area containing dioxin has still not agreed upon methods to be employed. Frustration at the apparent inactivity has been expressed both by experts anxious to begin the work of decontamination, and by former residents of the area who last week tore down protective fences in protest at the delay.

The work of the commission has been hampered by the disagreement among experts concerning the amount of dioxin to be dealt with. Dr Donald Lee of the Plant Pathology Laboratory at Harpenden, UK, suggests that 130 kg of dioxin was released in the explosion, and Professor Samuel Ep-

stein of the University of Illinois, considers the figure of 300 lb of dioxin to be "about right". But a group of chemical engineers invited by the commission to submit a programme for decontamination estimate that 1-3 kg of dioxin was released, a figure based on results obtained from a simulation of the explosion.

The two top officials of ICMESA, who after being arrested and charged with culpably causing a disaster were released on bail to supervise decontamination work within the ICMESA plant, have since been rearrested and criminal proceedings have begun. The criminal prosecution will be conducted at the same time as the civil proceedings brought against Hoffman-La Roche, ICMESA's owners, by the Italian authorities. □

EEC

Manoeuvring for agreement

The EEC's Council of Research Ministers met in Brussels last week to discuss once again the Joint European Torus (JET), the Community's fusion project, and the research programme for the Community's Joint Research Centre (JRC). Chris Sherwell reports

So near and yet so far. The EEC now looks sufficiently close to agreement on the site for JET that the small step the Council of Research Ministers took at their meeting last week may in retrospect come to look like a giant leap for the Community. But because there is still ample scope for more than one hitch, the prospect could be as much for a leap backwards as a leap for-

wards.

On paper the progress that everyone is keen to say really did occur in Brussels certainly looks very slight. The Council reached just one firm decision with any consequence in terms of action or expenditure. This was that the Community's fusion programme, which is undertaken by a number of associated laboratories in the member states but does not include JET, could go ahead for another four years.

To call that insignificant, though, is to reckon without the intricacies and nuances of Community deliberations. Until now there has been no agreement on expenditure for the full five years of the fusion programme. When the programme first came up for discussion

last December, Italy refused consent, saying she could not agree without a decision on a site for JET. She modified this position at a February Council meeting, allowing just one year's expenditure. In the view of one senior official in Brussels, therefore, the absence last week of continued further blocking of the programme from Italy means that the decision can be interpreted as a positive sign for JET.

What changed Italy's mind? The answer is to be found in the way the JET argument has developed. Italy's concern has always been principally over the fate of Ispra, one of the four establishments constituting the JRC—the others are in Germany, Belgium and the Netherlands, but Ispra is the largest with two-thirds of the JRC staff. By the last Council meeting in October it had become clear that progress on

JET might be possible if the Council would sanction in full the four-year (1977–1980) JRC research programme put up by the European Commission during the summer, much of which would go to Ispra.

The difficulty, however, was that Britain, France and Germany had made various demands for cuts in staff and expenditure on the proposed JRC programme. Germany was seeking cuts of less than 100 in staff, France was looking for cuts of around 200, and Britain had proposed a figure in excess of 300. Modifications in these demands was plainly necessary if there was to be any relaxation in the Italian demand for JET at Ispra—a demand which the Commission had itself backed on the grounds that a Community project should go to a Community centre.

The flexibility was evidently there, and the key development last week was a provisional agreement on these matters. Britain and France went to Brussels apparently prepared to pare back their demands to around 100—the number which it was thought could be lost through “natural wastage”—and eventually conceded along with Germany that the figure could be 80. This was the compromise proposal put up by the Commission after no agreement could be gained on the Commission's original compromise proposal of 50. It is damaging to Italy's hopes for Ispra inasmuch as the staff cuts, details of which obviously have to be finalised, are likely to fall most heavily on Ispra.

The agreement makes for a reduction in operating expenditure from 175 million units of account (mua) to 146 mua and so cuts the overall JRC budget for the four years to something like 346 mua. But there is a catch in all this: the agreement is conditional on

a suitable agreement being reached over JET. Thus, although the JRC research programme is at last final, little if anything can be done about it without further progress on the matter of the JET site—and on this there was little progress in Brussels.

What chinks of light there are, though, suggest that the matter is now delicately poised. Seven of the nine countries agreed last week that the site ought to be at a location with previous practical fusion and plasma physics experience. Significantly, it was France and Italy who thought this not to be relevant. France's proposed site, Cadarache, does not have fusion experience. Ispra has fusion experience and is not officially withdrawn, even though it now looks a less strong candidate with the provisional agreement on the JRC programme. The cases of both Culham in Britain and Garching in West Germany are strengthened by their experience.

The uncertainty is thus tangible. There are differences between France and the other countries on the degree of Euratom staffing that JET ought to have. But these are thought not to be insuperable. More importantly, the Council could not even agree last week on a procedure by which agreement might be reached. As a result, the Dutch chairman, Mr Brinkhorst, and the EEC Research Commissioner, Mr Brunner, are to tour the countries concerned to discuss the sites still further. If they determine that the basis for an agreement exists—the agreement when it comes will have to be unanimous—they will call for another Council meeting, provisionally scheduled for December 20.

The Community's fusion director, Mr Palumbo, has a few illusions about

that meeting, since if it takes place it would be the last one as far as JET was concerned. If it doesn't take place, he stresses, the whole project could be seriously endangered. Officials concerned with the JRC are somewhat more sanguine about the impact of a delay on the research programme, even though it is due to start in January. But the delay could be enormous. A new country takes the Council chair in January. That is Britain. And a new president takes over at the European Commission. He is from Britain. The latter change could well exacerbate any delay. Both changes could, in the present circumstances, make a difference to the outcome over JET.

No one, of course, is saying that the changes will help Culham win JET. But it was generally agreed last week that the choice had now narrowed substantially. In fact the case for Culham may well be strengthening. If Ispra is now discounted, the argument goes, that leaves Cadarache and Garching as Culham's competitors. And the rather sudden strength in the previously quietening voice for Cadarache can be discounted because it comes too late, because seven countries think experience is important and because Britain wouldn't wear it.

Garching can also be discounted, the argument goes on, because the Germans once again did not push strongly for it at last week's meeting, because it has the biggest fusion programme of the nine countries and plans its own large machine (AZTEC) anyway, and because it already has a Community research establishment. But if Germany does not get JET, what would it get? Well, speculate the cynics, watch out for a German filling the post of JET project director. □

BRITAIN

Changing the framework

A UK parliamentary committee last week published a 96-page report on university-industry relations. Chris Sherwell reports on the various institutional proposals of what could become a controversial document

WITH friends like the House of Commons Select Committee on Science and Technology, those amongst Britain's science community looking for a bit of stability in the country's science policy framework may not need enemies. For at the very time when the projected evolution of that framework seems virtually complete, the committee has published a wide-ranging report going beyond the narrowest confines of its

subject to offer proposals for further substantial changes.

The report is the result of written submissions and a score of public hearings initiated by the committee's Science Sub-committee. It follows an interim report on scientific research in British universities published in July 1975 and a second report released at the end of last year, and is “concerned with the purposes of the institutions of advanced scientific education and research”. The committee, clearly inspired by the idea that science policy should relate to the general social and economic objectives of the community, says it is essential “that we should be prepared to re-examine the organisation of science and scientific education in

terms of our current needs”.

Its recommendations regarding the framework within which British science policy is conducted may not throw the whole matter into the melting pot again. But in focusing directly on the country's capacity to conduct research and development they seem certain to fuel the arguments over whether an explanation of Britain's economic performance is to be found in the areas of innovation, investment and productivity, and hence also in the very platform of its science policy.

The most far-reaching recommendations are for the appointment of a junior minister in the Department of Education and Science (DES) with responsibility for science, for the transfer of funds from the Science Research Council (SRC) to the Department of Industry (DoI), for a review of the

relationship of basic and applied research in Britain, and for a possible new institution to replace the National Research Development Corporation (NRDC).

The proposal for a minister emerges from a discussion of education and training of engineers and applied scientists, for which the report argues most strongly. Among other things the committee also says the concept of Special Institutions for Scientific Education and Research (SISTERS) should be "revived and implemented", and urges the DoI and SRC to devote greater attention to the "teaching company" idea—a recommendation which post-dated by a couple of days an announcement of a £500,000 scheme for three such companies to improve university-industry links. But the idea of a Science Minister will look curious to many, and not just because it is an old idea that was dismissed when the present policy framework was worked out.

The minister, the report says, "should be principally concerned with scientific and technical education at all levels of the education system, and with the activities funded from the Science Budget". That seems unlikely to find much favour among those who relate the need for a minister to the amount of money he would handle, and still less likely if the biggest money-eater in the Science Budget, the SRC, ought according to the committee to have some of its funds transferred elsewhere.

The argument for that transfer—perhaps the most contentious proposal—is straightforward. The direction of a country's basic research, it goes, affects its capacity to do applied research which is geared to national needs. Traditional academic criteria directing basic research cannot guarantee that capacity, even with unlimited money; with declining funds, a research council system which uses such criteria is a luxury. Knowing this, the argument goes on, the research councils talk in terms of national needs and are put in an intolerable position; the SRC, whose support for applied research (unlike other research councils) is not subject to the ameliorating effects of the customer-contractor principle, suffers especially.

Thus, proclaims the report, "there is a good case for the transfer of a proportion of the Council's funds to the Department of Industry, which is the natural 'customer' department for the applied research supported by the SRC". The idea is to let the SRC take the decisions it is qualified to take, and not review research needs in terms of inadequately-defined national priorities. Those priorities should be determined by those responsible—the commissioning departments acting through their

ministers in Cabinet.

The proposal for a review of the relationship of basic and applied science is similarly related to the existing framework of science policy. In the three years up to April 1976 during which funds previously disposed of by research councils were transferred to the relevant departments under the customer-contractor arrangements, other organisational changes went ahead. Departments acquired chief scientists, and the emerging task of coordination between departments fell to an inter-departmental committee of chief scientists and permanent secretaries. The position of Chief Scientific Adviser to the government fell away, but the Cabinet's Central Policy Review Staff in turn had a chief scientist attached to it.

In addition another entirely new body was also created. Known as the Advisory Council for Applied Research and Development (ACARD), it was designed as the equivalent in applied research to the Advisory Board for the Research Councils (ABRC) in basic research; it is chaired by the Lord Privy Seal. Welcoming ACARD, the committee says it should "review the relationship between government-supported applied R&D and government-funded basic research as a matter of urgency". In particular it should "examine the operation of the customer-contractor relationship and of the ABRC to ensure that effective machinery exists for relating basic science policies to long term departmental R&D strategies". ACARD's reviews should be published, and the Lord Privy Seal ought also to make annual reports to parliament, the committee says. It does not consider whether ACARD is the appropriate body to conduct such examinations.

The committee claims it is not making sweeping recommendations for changes in the organisation of government R&D. Among its other proposals is one that the government undertake "a thorough review of the level and nature of the research undertaken in their own establishments" and attempt to transfer to universities and polytechnics "work of a more basic nature, not requiring major physical research facilities, wherever this is possible". Another is that encouragement be given, for example, to bringing higher education and industry into closer alignment; the committee says there is a good case for devising financial incentives "possibly in the form of generous tax allowances" to encourage companies to place research contracts with universities.

The most controversial points it makes in this area, though, are likely to be those concerning the NRDC. The committee says "urgent attention"

should be paid by the government to the "mis-match between the activities of the SRC and NRDC", and "urgent action" should be taken to correct it. It recommends a number of changes which, if implemented, would make the NRDC less concerned with producing a financial return from the results of research and more able to give advice and assistance. The functions it proposes for NRDC, says the Committee, may well be better performed "by a new institution without the accumulated scepticism and indifference which NRDC's policy and activities appear to have generated in some quarters"; as it is, the NRDC activities are "in no way conducive to encouraging the exploitation of academic research".

Last week the head of NRDC, comparatively unexcited by the report, said he would have been surprised if there hadn't been some adverse criticism, particularly as the committee provided an ideal forum for complaint. He happily acknowledged that there was some truth in the report's comments on NRDC but thought many of the criticisms were unjustified, adding that NRDC expenditure in relation to higher education institutions would have to be perhaps quadrupled if the right service was to be given, and more than a simple change in the NRDC's terms of reference was involved.

The SRC had not by the beginning of this week made any plans to issue a statement on the committee's report. Whatever the reaction to it inside universities and industry, it seems clear that its real impact will depend ultimately on how the Select Committee itself is viewed as an institution. The committee's members are hoping now for a parliamentary debate on the subject, which seems the only viable means by which its recommendations can be urged upon the government short of some positive response from outside. □

● A team of about 10 scientists would be adequate for British biological warfare research, a junior Defence Minister, Mr Gilbert, said in a parliamentary written reply last week. A reduction in military research in the Microbiological Research Establishment at Porton Down, Salisbury, had been on the cards since the announcement of a review of its spending in the March 1976 White Paper on Defence. Mr Gilbert's announcement means that the establishment's future depends on its civil work, at present under study by the government's Central Policy Review Staff and the National Institute for Biological Standards and Control, who hope to report before the end of the year. Up to one third of the establishment's running costs are currently met by revenue from its civil work.

news and views

Intermittent nerve conduction

from Shin-Ho Chung

ALMOST every axon in the central nervous system ramifies profusely before making synaptic contacts with its target cells. This terminal arborisation is formed by the axon bifurcating into branches, the branches into twigs, and the twigs into fine terminal filaments. A single axon entering the superior cervical ganglion, for example, establishes about 1,500 synapses with its post-synaptic neurones, and all of these contacts are believed to be activated by an impulse in the main axon. However, several recent studies have shown that not every impulse flowing down the main axon invades all of its terminals; instead, at each bifurcation some impulses are channelled into one branch and are prevented from invading the other. This process, known as 'intermittent nerve conduction', is incompatible with the classical idea of the axonal tree as a simple distributor of nerve impulses to a large number of the next-order neurones, and it invites further investigation.

Barron and Matthews (*J. Physiol. Lond.*, **85**, 75; 1935) first noticed the phenomenon of intermittent conduction in a class of sensory fibres in the cat, which enters the spinal cord and sends branches back to the skin through neighbouring dorsal roots. When monitored in a branch coming out of the cord, a steady stream of impulses entering the cord was found to be transformed into an intermittent series, with an alternating sequence of discharge and silence. The pattern of this conduction block depended on the frequency of discharge in the recording fibre as well as on the amount of neuronal activity in the neighbouring fibres; for example, when the spinal cord was active with ascending sensory discharges, the block became frequent and long-lasting. Barron and Matthews believed that this phenomenon of intermittent conduction was one of the mechanisms by which the central nervous system regulates the flow of impulse traffic and 'transforms

a simple sensory discharge into a sensation.' After the dorsal root reflex was described (Toennies, *J. Neurophysiol.*, **1**, 378; 1938), Barron and Matthews' observations were erroneously assumed to be a special instance of it, and, except for a few sporadic notes (Fuortes, *J. Physiol. Lond.*, **112**, 42p; 1950; Howland *et al.*, *J. Neurophysiol.*, **18**, 1; 1955), their very accurate and detailed descriptions were, in the main, ignored.

The phenomenon has now been re-examined in the simple nervous system of invertebrates, where the branching pattern of a single axon can be viewed under a microscope. In the crustacean leg or abdomen, for example, a single motor neurone branches many times in its intramuscular course, and the terminals of the two main branches innervate two separate groups of muscles (Bittner, *J. gen. Physiol.*, **51**, 731; 1968). When the main axon is stimulated at a low, steady frequency, impulses travel to both groups of muscles, but the junctional potential is large enough to cause contraction in only one of them. As the frequency of stimulation increases, the speed of propagation into the branch serving the contracting muscle becomes reduced as the impulse momentarily hesitates before invading it (Hatt and Smith, *J. Physiol. Lond.*, **259**, 367; 1976). The flow of impulses into this branch is then abruptly blocked, whereas the other branch continues to admit impulses and the muscle served by it begins to contract (Grossman *et al.*, *Brain Res.*, **64**, 379; 1973; Parnas, *J. Neurophysiol.*, **35**, 903; 1972). Hints of a similar phenomenon exist in the results of studies on a variety of other neurones such as the sensory afferent in the leech (Van Essen, *J. Physiol. Lond.*, **230**, 509; 1973) and the giant axon of the cockroach (Spira *et al.*, *J. Neurophysiol.*, **39**, 882; 1976; Castel *et al.*, *ibid.*, 900).

The mechanism by which the propagation of nerve impulses is blocked

is not thoroughly understood. There are several reasons for supposing that the conduction failure occurs at the point of branching or at the region where the geometry of the axon undergoes a sudden change. Ordinarily, the currents produced by an impulse travelling down an unbranched axon have about four times the magnitude necessary to ensure continuous invasion. At points of branching, the current from the parent axon is split between the daughter branches and, at the same time, must charge a larger capacitance. This causes not only an attenuation of the exciting current, but also a hesitation of the nerve impulse, allowing sodium inactivation to develop. Such a region with a low safety margin is likely to be most susceptible to a small shift in ionic environment, and accumulation of some ions in the extracellular space following repetitive stimulation may further reduce the safety margin so that the transmission of nerve impulses fails completely (Smith and Hatt, *J. Neurophysiol.*, **39**, 794; 1976; Spira *et al.*, *op. cit.*). Theoretical computations based on the Hodgkin-Huxley equations predict that the increased concentration of potassium in the extracellular space during repetitive firing will cause conduction failure at the region where the geometry of the axon undergoes a sudden constriction (Parnas *et al.*, *J. Neurophysiol.*, **39**, 909; 1976). This, however, is not the only condition which may bring about the conduction block. Prolonged hyperpolarisation of the axon (Van Essen, *op. cit.*), temporary anoxia (Krnjevic and Miledi, *J. Physiol. Lond.*, **149**, 1; 1959), or currents arising from activity of neighbouring fibres (Lettvin *et al.*, *Brain Behav. Evol.*, **3**, 72; 1970) may have the same effect.

Whatever the precise mechanism ultimately turns out to be, the finding that axonal trees do not faithfully convey impulses to their terminals will have several important implications in

neurobiology. If we accept the classical view of the axon as a passive distributor of impulses, then there appears to be a serious defect in its performance. In order to overcome this defect, the nervous system may form a large number of redundant connections so that intermittent failure of nerve conduction will not seriously hinder the processing of neural information. Alternatively, as Lettvin and his colleagues maintain (*Brain Behav. Evol.*, *op. cit.*: see also Waxman, *Brain Res.*, **47**, 269; 1972), the

axonal tree may act as a filter, regulating the flow of impulses into one or other branch, depending on the temporal pattern of firing. It is quite common for a single nerve to innervate more than one type of post-synaptic cell, and if the axon is capable of controlling one or the other, or both, through its firing pattern, as demonstrated in the crustacean motoneurone, then what appears to be a fatal defect of the bifurcating axon may turn out to be a very useful property in achieving an economy in neural organisation.

Mysteries of early dinosaur evolution

from Barry Cox

ALTHOUGH many pages have been written discussing the mystery of the extinction of the dinosaurs, almost as much uncertainty surrounds their origin—or origins. The large Mesozoic archosaurian reptiles known as dinosaurs belong to two separate groups, the Saurischia and the Ornithischia, which differ in such characters as the structure of the pelvic girdle and the possession of a prenatary bone (present only in ornithischians). A diversity of saurischians is known from the late Triassic, as are about six genera of ornithischians. But the relationships of the two groups to each other, or to the earlier Triassic archosaurians known as pseudosuchians, remain uncertain.

By the Early Jurassic, saurischians include at least two types. The small carnivores (or coelurosaurs) and the large carnivores (or carnosaurs) were both bipedal, and are both placed in a single group, the Theropoda. Late Triassic coelurosaurs are known, but no Triassic carnosaurs have yet been found. The other saurischian group, the Sauropoda, comprises the very large, quadrupedal, long-necked herbivores of the Jurassic and Cretaceous. Their Triassic ancestry runs back into an assemblage of quite large facultatively bipedal forms, the prosauropods, which include both herbivorous and carnivorous types. It is therefore at least possible that the coelurosaurs, the carnosaurs and the prosauropods all had quite separate origins from within the pseudosuchians. Various authors, such as Charig and Bonaparte, have suggested this, basing their views mainly on apparently divergent structural modifications of the ankle in these groups.

So far, at least, there has been less evidence of diversity in the Triassic ornithischians; only two, rather similar families are known. Smaller than most of the saurischians, their structure has

been less well known, but an article by Santa Luca, Crompton and Charig in this week's issue of *Nature* (page 324) provides a preliminary account of an exceptionally fine specimen from the very late Triassic of South Africa. This little bipedal, fast-running herbivore, *Heterodontosaurus*, had already developed a number of specialised features, such as the grasping hand and some degree of fusion of the bones of the ankle. Ornithischians, then, had also clearly been evolving and radiating for some time before the end of the Triassic.

If one now attempts to relate these two types of dinosaur to the Triassic pseudosuchians, there appears to be a puzzling overlap in time between the two groups, although possible evolutionary links between them obstinately refuse to appear. Though the pseudosuchians include large bipedal carnivores, the ornithosuchids of the Middle and Late Triassic, which have been suggested as ancestral to the carnosaurian dinosaurs (Walker, *Phil. Trans. R. Soc.*, **B248**, 53; 1964), this has since been denied by Bonaparte (*Coll. int. Cent. nat. Rech. sci.*, **218**, 485; 1975). The large Late Triassic quadrupedal pseudosuchian carnivores known as rauisuchids (or prestosuchids) have been nominated as the possible source of the saurischians, while the only pseudosuchian similar to the little ornithischian dinosaurs is the small bipedal genus *Euparkeria* of the Early Triassic.

The possible ancestors of the different types of dinosaurs are thus scattered through the Triassic, and the pseudosuchians persisted until the end of that Period. Throughout the Late Triassic, at least, a variety of pseudosuchians therefore coexisted with a variety of their presumed descendants, the dinosaurs, as has been pointed out by Sill (*Bull. Mus. comp. Zoo. Harv.*, **146**, 317; 1974). The perhaps multiple

evolution of dinosaur lineages therefore did not lead to the rapid ecological replacement of their pseudosuchian ancestors. These instead persisted, together with their relatives the semi-aquatic phytosaurs and the pig-like rooting aëosaurs, until the end of the Triassic, when all became extinct together—though no new ecological replacements for them appeared at that time.

So the poor palaeontologist searching for answers is therefore, in the origin of the dinosaurs, confronted with complexity where he hoped for simplicity, while in the replacement of the pseudosuchians by their varied offspring he meets a sudden (if delayed) simple event where he expected complexity! Today, the fashionable *deus ex machina* for explaining extinctions is continental drift, but even rising sea levels resulting from the beginning of the opening of the Atlantic in the Late Triassic seem unlikely to provide a solution to these puzzles. □

Future of the CERN intersecting storage rings

from M. G. Albrow

A Workshop on Future ISR Physics was held at CERN, Geneva on October 4–15, 1976.

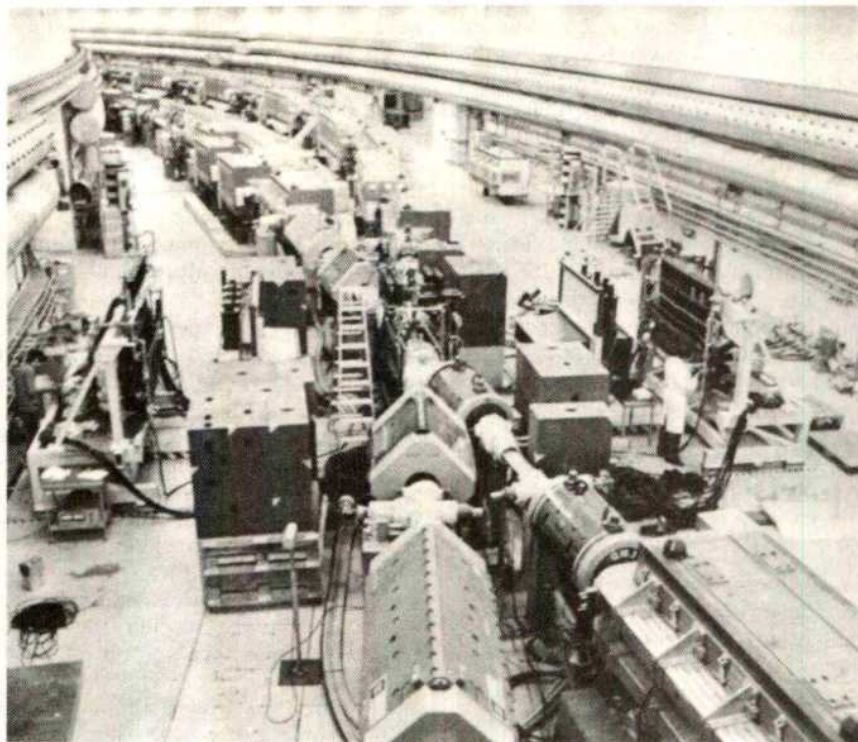
In January 1971 two beams of high energy protons were trapped in the CERN Intersecting Storage Rings (ISR) and brought into head-on collision for the first time. This achievement enabled high energy physicists to study particle interactions, under well-controlled laboratory conditions, at energies otherwise accessible only with cosmic rays. The ISR beam energy ranges up to 31 GeV; an accelerator of 2,000 GeV would be needed to obtain the same effective collision energy using a particle beam on a stationary target. The present commissioning of the 400 GeV SPS accelerator at CERN, and plans at the Fermi National Accelerator Laboratory to construct a 1,000 GeV "Energy Doubler", have stimulated a re-evaluation of the role of the ISR in high energy physics. There is no doubt that the ISR has a unique position in terms of available collision energy; the question is whether that advantage is important enough to guarantee an exciting programme of physics for many years to come, and therefore to justify its continued operation in the present financial climate. A workshop, attended

by about 50 physicists from Europe and the United States, was recently held at CERN to discuss such questions and try to define a research programme for the ISR after the present generation of experiments, that is, into the next decade. Here I have attempted to summarise the most promising of the ideas considered.

The concept that the strongly-interacting particles (or hadrons) are composite objects containing more fundamental constituents, for example quarks and antiquarks, has achieved almost universal recognition over the past 12 years. All the known hadrons can be constructed from a basic set of four quarks q (up, down, strange and charmed) and their four antiquarks \bar{q} . The proton is supposed to contain three "valence" quarks (up, up and down) which determine its quantum numbers, as well as a sea of $q\bar{q}$ pairs and particles called gluons which bind the quarks together. In this picture, when two protons pass through each other the point-like quarks usually miss each other—they may however get shaken up with excitation energy. These excited protons then break up into normal ground state hadrons. Occasionally a $q\bar{q}$ or a $q\bar{q}$ collision occurs, and because of their point-like nature a large angle scattering is likely. The scattered quarks themselves do not emerge as free particles, but "dress up" into normal hadrons which frequently have large momentum components transverse to the collision axis. The discovery at the ISR of relatively large numbers of hadrons with large transverse momenta supports this concept of point-like hadron constituents, just as the observation of large angle α particle atom scattering led Rutherford to discover the atomic nucleus.

However much more data are needed on this rather rare type of collision to establish the precise nature of quark-quark scattering, and the next generation of experiments probably requires a large new particle detection system, which would allow the identification and momentum measurement of most of the particles created in the collisions. The importance of the ISR in this field is that these processes have a very strong energy dependence and are therefore less amenable to study in lower energy machines.

Many of the present experiments at the ISR concentrate on lepton production. Four leptons are known (e^- , μ^- , ν_e , ν_μ and their antiparticles). It may be more than a coincidence that we know of four quarks. The leptons do not interact strongly and should only be produced indirectly in hadron-hadron collisions, for example through the production and decay of



One of the intersection regions of the ISR showing the point (centre) at which the two regions cross.

other particles (such as charmed hadrons, see *News and Views*, **262**, 537; 1976) or through $q\bar{q}$ annihilation:

$$q\bar{q} \rightarrow \gamma \rightarrow \mu^+ \mu^-$$

where γ is a virtual photon or a heavy photon-like particle, for instance the J/ψ . (Samuel Ting and Burton Richter were awarded the 1976 Nobel prize for physics for the discovery of this particle, see *Nature*, **263**, 714; 1976.) Current experiments are searching for new J/ψ -like particles and, if the annihilation mechanism is correct, are able to map out the q and \bar{q} distributions inside the proton. Many physicists expect new surprises in this field; if so the research programme is completely open ended.

The high energy (and wide energy range) of the ISR is also vital for the study of some other, perhaps less fashionable, processes. I mentioned earlier the possibility of one proton becoming excited into a higher energy state in a collision; the other proton may recoil in its ground state acting only as a "spectator". The energy or mass of these excited states grows linearly with the effective collision energy, and reaches values of order 12 GeV at the ISR (compared with about 3–4 GeV at the SPS and Fermilab). Little is known about these massive states, for example their composition in terms of particle type. A related process is expected to "turn on" in the ISR energy range, in which both incident protons emerge un-

changed from the collision except for a small loss of energy. The energy lost by the protons reappears in the form of hadrons which essentially "pop out" of the vacuum (the vacuum is supposed to be full of virtual particle pairs which can materialise if sufficient energy is supplied). If this process does not exist several theoretical ideas must be incorrect; if it does exist it is rather fundamental and should be studied in depth.

The ISR Workshop also discussed possible improvements to the machine itself: higher collision rates, other particles in the beams (antiprotons, deuterons and heavier ions) and higher energy beams. Beams of 120 GeV would be possible if the present magnets were replaced with 5 Tesla superconducting magnets, giving an energy equivalent to that of a 30,000 GeV accelerator. This is clearly a possibility for longer term development, and in my view a most exciting one. Theories which unify the electromagnetic and weak forces predict three very fundamental particles (W^\pm , Z^0) which are responsible for the weak interaction (radioactivity) with masses near 65 and 80 GeV. They would probably be produced at the ISR if it were upgraded in this way, and much of the other physics studied at present would greatly benefit from an extension in energy.

I believe most of the participants left the Workshop with considerable enthusiasm for the future possibilities of the ISR. First, there are many questions about very high energy inter-

actions that need answering, and which appear to be answerable with the machine as it is today, but are beyond the range of lower energy machines. Second, improvements to the machine and the detectors will widen the scope of these investigations, and it is not unreasonable to suppose that new surprising discoveries will be made. Finally the investment made in the ISR has given Europe a unique facility which could be made a stepping-stone into a completely new energy region. \square

Pair-breaking in superfluid ^3He

from P. V. E. McClintock

STUDIES of the way in which negative ions move through liquid ^3He have resulted in a particularly revealing demonstration of the superfluid properties which it exhibits in the temperature range around 1 mK. The experiments, carried out at Helsinki Technical University by A. I. Ahonen, J. Kokko, O. V. Lounasmaa, M. A. Paalanen, R. C. Richardson, W. Schoepe and Y. Takano, and reported in *Physical Review Letters* (37, 511; 1976), represent the first successful investigation of ion motion in the recently discovered superfluid phases of the liquid.

The so-called ion formed when an excess electron is injected into liquid helium is actually a semi-macroscopic spherical structure with a diameter of around 3 nm and an effective mass about a hundred times greater than that of a helium atom. Being negatively charged, ions may be directed by means of externally applied electric fields and their arrival at a collecting electrode may be detected as a current. They therefore constitute particularly convenient probes for elucidating the behaviour of the liquid, and they have already been employed to excellent effect in gaining insights into the nature and fundamental properties of superfluid ^4He . Comparable investigations of superfluid ^3He are, of course, considerably more difficult because of the exceedingly low temperatures which must be achieved and maintained: the superfluid transition temperature of ^3He is near 2 mK, or about a thousand times colder than that of ^4He .

The ^3He in the Helsinki experiment was refrigerated, using a nuclear demagnetisation cryostat, to temperatures less than half that of the superfluid transition. The measurements themselves, based on a technique recently developed at Lancaster University, were in essence exceedingly simple. Bunches of ions, injected into the liquid from a field emission tip,

were gated into a drift space of known length which they traversed under the influence of a fairly uniform electric field, finally arriving at a collecting electrode where they were detected as pulses of current. By measuring the time taken to cross the drift space, the characteristic velocity of the ions could thus be determined for a number of different temperatures and electric fields.

Some typical experimental results for the B phase (the form of superfluid ^3He which is stable at low pressure) are shown in Fig. 1. It is strikingly evident that, for any given electric field, the ions travel much faster as the liquid is cooled below the temperature T_c of the superfluid transition. By analogy with the behaviour of ions in liquid ^4He , it is natural to try and account for the general shape of these curves on the assumption that there are two separate types of mechanism by which the kinetic energy of a moving ion may be dissipated: (1) at all finite temperatures the ion travels through the residual gas of normal, that is, unpaired, ^3He atoms which tend to impede its progress; (2) if the velocity of the ion is high enough, additional dissipative mechanisms can come into play whereby the ion creates excitations in the liquid. On this model, the ionic drift velocity which is measured in practice represents an average over the numerous individual scattering and possible excitation creation events which occur during the transit.

For sufficiently small ionic drift velocities, where only (1) need be considered, the velocity-field characteristic is expected to be linear with its gradient defining the ionic mobility. It may be noted from the figure that this is indeed the case and that, as the temperature is reduced, the mobility increases rapidly, consistent with the expected decrease in scattering as more and more of the ^3He atoms fall into the paired states that comprise the superfluid. Indeed, at the lowest temperatures, the mobility apparently became so large that it was impossible to observe the linear region at all. Comparing these results quantitatively with theoretical predictions by T. Soda (in *Proc. 14th Int. Conf. on Low Temp. Phys.*, edit by Krusius, M. and Vuorio, M., 1, 13, North-Holland, Amsterdam, 1975) and R. M. Bowley (*J. Phys.*, C9, L151; 1976), Ahonen *et al.* point out that both of these calculations substantially underestimate the magnitude of the superfluid mobilities. Probably, however, we will not have to wait very long for the theorists to improve their calculations, now that they have the stimulus of some real experimental data.

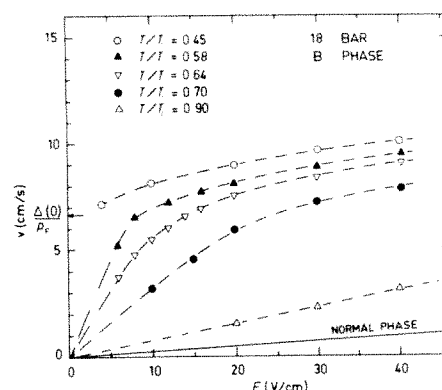


Fig. 1 The drift velocity v of negative ions moving through superfluid ^3He under the influence of an electric field E , for various temperatures. The behaviour in normal (non-superfluid) ^3He is shown by the full line, and the predicted value of Landau critical velocity for pair-breaking is indicated by an arrow.

Assuming that the observed bending over of the characteristics for velocities above a few cm s^{-1} is the result of mechanisms of type (2), it is natural to enquire into the nature of the excitations which are being created, and there are several possible candidates. For each of these there will be a so-called Landau critical velocity v_L , which the ion must exceed if the general requirement for simultaneous conservation of energy and momentum is to be satisfied during the creation process. At very low temperatures, where dissipation through (1) may be ignored, one would expect that an ion moving under the influence of a small electric field will accelerate without hindrance until it reaches v_L , and that it will then continue moving at a constant drift velocity equal to v_L while dissipating, in the form of excitations, the energy gained from the electric field. Thus, the position where the lowest temperature curve in the figure, that for $T=0.45 T_c$, cuts the velocity axis gives a value of v_L which can be used to try and deduce the nature of the excitations. The simplest assumption, by analogy with the superfluid electron gas in a superconductor which in many respects is a similar system, is that the excitations emitted by the ion are just unpaired ^3He atoms. The Landau critical velocity for breaking apart the pairs of ^3He atoms forming the superfluid may be estimated as about $v_L=6.7 \text{ cm s}^{-1}$, and is indicated by an arrow in the figure. The fact that the curve looks as though it would extrapolate back to cut the velocity axis very close to this point is thus of considerable interest and significance, implying that energy dissipation under these conditions is indeed through the anticipated pair-breaking mechanism, rather than through crea-

tion of the (vortex-like?) excitations which may have given rise to the much lower critical velocities observed in the flow experiment recently performed by R. M. Mueller, E. B. Flint and E. D. Adams (*Phys. Rev. Lett.*, **36**, 1460; 1976) at the University of Florida.

Although a number of important conclusions may be drawn from the present data, the Helsinki experiment—as the authors themselves point out—is really only a beginning. Their principal achievement is to have demonstrated the technical feasibility of using ions to probe the superfluid phases of ^3He . It is to be hoped that future experiments of this type, taken in conjunction with improvements in the theoretical situation, will enable the temperature dependence of the energy gap separating the paired atoms of the superfluid from the (unpaired) excited states of the liquid to be mapped out in detail; it should be possible, using a more carefully designed geometry, to explore the anisotropy of the A phase; and the use of positive ions, to judge from earlier experience in superfluid ^4He , may perhaps enable completely new types of excitation to be created and identified. □

Nitrogen aggregates in diamond

from John Walker

As regular readers of News and Views may remember, nitrogen is a common impurity in diamond, though how exactly it got there and the precise forms it adopts within the crystal are only poorly understood. But a recent paper by G. Davies (*J. Phys. C: Solid St. Phys.*, **9**, L537–L542; 1976) partly resolves the latter question. The question is important, and not only for scientific reasons since it bears on the synthesis of diamond.

Synthetic diamonds are usually yellow, because they contain a small amount (perhaps one part per million) of dispersed nitrogen. That is, each nitrogen atom is isolated from the other nitrogen atoms, and replaces one carbon atom in the diamond lattice. In the current classification these are type Ib diamonds. Natural diamonds of this type are rare—one in a thousand.

The majority of natural diamonds, and all the large, well-formed ones suitable for adorning sceptres and film stars' fingers, are what we call type Ia stones. They also contain nitrogen impurity, in quite large proportions (up to almost one per cent); but in contrast to type Ib the nitrogen is aggregated. It appears that in growing her diamonds

Nature did not use the same process as General Electric and De Beers (fortunately for the gem industry, since yellow diamonds, though quite pretty, lack the sparkle and fire of water-white stones).

So what is the form of these aggregates? Until a couple of years ago we thought we knew the answer. The nitrogen gathered in large clusters, many thousands of angstroms in diameter, along {100} planes—the "platelets" that Evans and Phaal (*Proc. R. Soc. Lond.*, **270**, 538–552; 1962) had discovered by electron microscopy. Unfortunately we were wrong. We do not know what the platelets are, but thanks to earlier work by Davies (*Nature*, **228**, 758; 1970) and by E. V. Sobolev *et al.* (*Sov. Phys. Dokl.*, **12**, 665–668; 1967) we know that they contain little if any nitrogen.

We do know that there are at least two different types of aggregate, the so-called A and B forms, which give rise to characteristic infrared and ultraviolet absorption. What Davies has done in his most recent paper is to study one of the A bands, in fact a sharp line, under uniaxial stress. That is, he has squeezed a rectangular diamond block between two anvils, at stresses of the order of 20,000 atmospheres. As Wedlake has remarked, for diamond this is only a romantic squeeze; but it can nonetheless produce interesting results. Although the diamond lattice is cubic, under stress its symmetry is reduced (to tetragonal, trigonal or rhombic depending on the direction of stress), causing absorption bands to split into two or more components (just like the Zeeman effect in atomic spectroscopy—the splitting of spectral lines by a magnetic field).

Davies has deduced from the splitting pattern that the defect responsible for the A form of nitrogen has trigonal symmetry. The simplest atomic arrangement consistent with this is a pair of nitrogen atoms next to each other ("nearest neighbours"), replacing a pair of carbon atoms in the diamond lattice. Such a model fits in with the other constraints placed by current experimental knowledge: it is not paramagnetic, it is too small to be detected by electron microscopy, and it accounts for the slight increase in lattice parameter observed in nitrogen-bearing diamonds compared with pure specimens.

In a further paper (*Proc. R. Soc. Lond.*, **A351**, 245–265; 1976) Davies and his colleagues, again using uniaxial stress, demonstrate that the well-known H3 absorption and luminescence system in irradiated and annealed diamond has rhombic I symmetry. Davies (*J. Phys. C: Solid St. Phys.*, **5**, 2534–2542; 1972) has already shown that the H3 defect is produced when the A form of nitrogen traps a vacancy or interstitial

carbon atom produced during irradiation. Rhombic symmetry follows neatly from the trigonal symmetry of the A aggregate.

What we do not yet know is did the nitrogen aggregate into pairs by diffusion, or was it created like that during synthesis? What is the atomic arrangement of the B aggregate, and is nitrogen involved in the formation of the platelets? We still have many questions left to answer. □

Electron transfer systems in microorganisms

from D. O. Hall

An International Symposium on Electron Transfer Systems in Microorganisms was held at the CNRS Laboratory of Chemical Bacteriology, Marseilles on November 2–5, 1976.

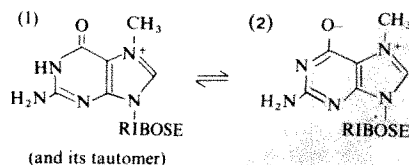
THE Marseilles laboratory has been active in the biochemistry of electron transfer in anaerobic bacteria for many years and the meeting was held to discuss the requirements of electron transfer at both the cellular and the molecular level.

The genetics of yeast metabolism has been a fruitful subject for research for some time. P. Slonimski (Laboratory of Molecular Genetics, Paris) discussed some of the recently discovered mitochondrial mutants (MIT⁻) which result in deficiencies in various aspects of mitochondrial metabolism. More than a thousand mutants are now available, opening up the possibility that mitochondrial functions for many different electron transfer reagents and enzymes will be available to biochemists to study the details of electron transport and ATP formation.

B. Haddock (University of Dundee) described his work on electron transfer systems in *E. coli* and *Paracoccus denitrificans*. It is becoming clear that

Correction

In the article "Eukaryotic mRNA: trouble at the 5'-end" (*Nature*, **263**, 188; 1976) the formula on page 190 was printed incorrectly. The correct formula is given below.



bacteria possess an electron transport chain containing many of the components familiar from mitochondria, such as flavoproteins, iron-sulphur proteins, ubiquinones and cytochromes. Since these bacteria are simpler to work with we now have, for example, a means of studying proton translocation to verify aspects of the Mitchell chemiosmotic theory and also to look at the role of compounds such as cyclic AMP in determining alternative pathways of electron transfer. Both these organisms can be grown in anaerobic and aerobic conditions, with precisely specified nutritional conditions, sulphate or iron limitation for example, which will affect components of the electron transport chain.

The occurrence of the enzyme superoxide dismutase to protect organisms against the harmful effect of the superoxide which is produced when oxygen interacts with free radicals is now a very active field. Only recently have anaerobic organisms been shown to contain superoxide dismutase. C. Hachikian (CNRS, Marseilles) reported on the isolation and purification of superoxide dismutase from the sulphate-reducing organism *Desulphovibrio*, an obligate anaerobe. This enzyme has iron in its active centre and is similar to the superoxide dismutase from aerobic prokaryotes, such as *E. coli* and the blue-green alga *Spirulina*. One of the hypotheses put forward as to why anaerobes need such an enzyme is that so-called anaerobic organisms need to be tolerant to small quantities of oxygen. The superoxide dismutase may be inherent to these organisms, or may have been acquired from an aerobic organism by plasmid transfer.

I. Probst (Göttingen) reported on the interesting symbiotic relationship between the sulphate-reducing, anaerobic bacterium *Desulphuromonas* and a green, sulphur photosynthetic bacterium *Chlorobium*. *Desulphuromonas* can utilise acetate as its electron donor and sulphur as its electron acceptor; the net energy difference in this reaction is rather small, but is quite sufficient for the organism to grow in symbiotic association with green photosynthetic bacteria. The green photosynthetic organism *Chlorobium* uses CO_2 , in a classic photosynthetic mechanism, with H_2S as its electron donor, and produces sulphur, which is then used by *Desulphuromonas* with acetate as its carbon source to produce CO_2 and H_2S thus completing the cycle.

The recent discovery of three different ferredoxins was reported by A. Xavier and J. Moura (Lisbon Institute of Science and Technology) from *Desulphovibrio gigas*. These ferredoxins exist as trimers or tetramers and interestingly enough are able to catalyse electron transfer at negative

and positive redox potentials in states of oxidation similar to those of the so-called "C-states" of Carter. What Xavier and Moura have shown is that naturally occurring 4Fe-4S proteins are able to exist as oligomers which themselves can give variations in redox potential. If it is a natural phenomenon and not simply induced by association of monomers *in vitro*, this may be the way in which iron-sulphur proteins can alter their redox potential either within a specific monomer or within the molecule as a whole by association of oligomers; oligomers, of course, can be induced by alterations in salt concentration, pH, and so on. This may in fact be one of the ways in which complex iron-sulphur proteins like nitrogenase and hydrogenase may be able to accumulate a number of pairs of charges before they catalyse reactions such as nitrogen fixation and hydrogen evolution. □

Control of external radiation dose

from a Correspondent

An international meeting sponsored by the Society for Radiological Protection and the UKAEA was held on October 19, 1976, at AERE Harwell.

THE protection of the environment has been widely discussed over the past few months. The objective of this meeting was to consider the main sources of occupational exposure which come from external β , γ and neutron radiations. J. A. Dennis (NRPB, Harwell) discussed the concept of dose-equivalent index and related topics. He expressed the personal opinion that the present lack of guidance from the International Commission on Radiation Units on the use of surveying parameters could give rise to overexposures. It is necessary to measure the dose equivalent at different depths in the body and the NRPB has proposed depths of 5 to 10 mg cm^{-2} and 700 mg cm^{-2} for skin and body respectively. J. R. Harvey (CEGB, Berkeley) was keen to introduce the concept of dose equivalent ceiling to avoid any possibility of overexposure. Thus it would be possible that a survey would give a potential dose of 200 mrem in a particular situation whereas measurements with personal dosimeters would give 100 mrem, both being equally valid, and it is on the latter dose that ultimate control would be exercised.

F. W. Spiers (University of Leeds) described the work by himself and T. Ashton to measure the dose to different organs in a man phantom, exposed to isotropic γ -ray sources (energy range 0.05 to 2 MeV). During discussion Spiers suggested that the dose to the active bone marrow was a reasonable approximation to the whole body dose and is approximately the dose under 53 mm of tissue. He also stated that the response obtained by rotating a phantom in a beam produces different results from those obtained by the use of isotropic radiation.

The large differences in shielding required by nuclear reactors and high energy accelerators was brought out by two papers by A. F. Avery (AEA, Winfrith) and G. R. Stevenson (CERN II, Switzerland). The accelerator shielding consists of earth and masses of iron (100 m long by 2 m in diameter) to bring the dose rate down to 1 mrem h^{-1} for occupational locations and 85 $\mu\text{rem h}^{-1}$ at the site fence. Nuclear reactor shielding is required to reduce the dose rate to 0.1 mrem h^{-1} in these areas, but of course far less material is needed.

In contrast to these two papers E. J. Henshaw (Radiological Protection Centre, Liverpool) said radiation levels up to 1 rem h^{-1} were common in fluoroscopy, but these levels are only present for brief periods. Henshaw suggested that it was important to measure the X-ray generator parameters, in particular the potential across the tube. During the discussion it was suggested that it would be of interest to do a comparative cost-benefit analysis between reactor shielding and dose control in radiology departments.

H. W. Julius (TNO, Netherlands) contrasted the film dosimeter and the thermoluminescent dosimeter (TLD) for personal dose control. The former provides much more detailed information but the TLD has advantages of low atomic number, and linearity of response with fewer environmental effects. J. A. Douglas (AERE, Harwell) described some of the work of Cavallini from Italy and his own studies on the limitations of the albedo technique for personal neutron dosimetry. His work had showed that the ratio of the incident to reflected flux of neutrons could not be used to give the energy of the neutrons except in certain circumstances which could apply for a limited range of radiation shields. J. R. A. Lakey (Royal Naval College, London) brought the meeting to a close by discussing what we do when we cannot reduce the dose rate below 2.5 mrem h^{-1} . He emphasised the importance of working schedules and the sharing of the dose between workers. □

articles

Slip-line field theory and large-scale continental tectonics

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A simple analogy is made between the tectonics of Asia and deformation in a rigidly indented rigid-plastic solid. India is analogous to the indenter and the great strike-slip faults correspond to slip lines. For various indentation geometries, the sense and linearity (or curvature) of strike-slip faults, convergence at the Burma arc and the existence of the Himalayan Burman Syntax, the conjugate strike-slip faults in Mongolia and the extension at the Baikal and Shansi graben can be predicted. Given the horizontal force necessary to support Tibet, an average shear stress of a few to several hundred bars along faults in Asia is predicted, corresponding to the yield stress of rigid-plastic material.

THE pattern of faulting in Asia, as revealed by analyses of Landsat photos and of seismic data (Fig. 1), bears a strong resemblance to the slip-line field calculated^{1,2} and observed³ for plane indentation of a plastic medium by a rigid die⁴⁻⁶ (Fig. 2). The analogous pattern of deformation for these two seemingly unrelated situations suggests that the continuum approach and the physical understanding of indentation of plastic materials, developed by mechanical engineers, could be used to gain further insight into the process by which continents deform when they collide with one another.

Rigid-plastic materials and plane strain slip-line fields

A plastic material is often assumed to be homogeneous, isotropic and perfectly plastic. There is no volume change and no strain hardening. A large class of problems, and those of interest here, assume a state of plane strain for which the two common criteria for the onset of plastic flow, or yielding (Tresca's and Von Mises's), are equivalent. Yielding occurs when the maximum shear stress, $\tau = (\sigma_1 - \sigma_3)/2$, reaches a limiting value, the yield stress, τ_y , where σ_1 and σ_3 are the maximum and minimum compressive stresses, respectively. Because of the mathematical difficulties in matching boundary conditions across the boundary between regions of elastic and plastic deformation and because the deformation in the plastic region is much greater than that in the elastic region, the elastic region is often treated as a rigid solid. Hence the material is said to be rigid-plastic: plastic where the yield stress is reached and rigid elsewhere. Figure 3a shows the stress-strain curve for such a solid.

All analyses are made at the yield point, so that $\tau \leq \tau_y$ throughout the region which deforms plastically. In this plastic

region the slip lines correspond to the maximum shear stress trajectories along which $\tau = \tau_y$. The mean (hydrostatic) stress, $\sigma_h = (\sigma_1 + \sigma_3)/2$, changes along the slip lines when they are curved, in a simple manner that can be determined from the equilibrium (Hencky) equations. Thus, from these equations, the geometry of the slip lines and the stress boundary conditions, the complete stress field in the plastic medium can be obtained easily.

Pure shear occurs on and parallel to the slip lines. They mark lines across which the tangential component of displacement can be discontinuous, as at faults in the Earth. The two types, α and β lines, differ only in sense, and in geologic terminology are right- and left-lateral respectively. As slip lines are lines of maximum shear stress, they must intersect each other at right angles and all traction-free boundaries at angles of 45° .

Solutions to rigid-plastic problems can be divided into those of steady and of unsteady flow¹. For steady flow, the boundaries do not change shape or relative position, and the pattern of flow is independent of time. The drawing of wire is an example. For most tectonic problems, however, it appears that solutions only for unsteady flow are of interest. Again two types of solution can occur. In some cases, the deformation occurs in such a way that, although the region of deformation increases with time and, hence, the boundary conditions change continuously, the geometry of deformation changes only in scale. An example is the indentation of a rigid-plastic medium by an infinite rigid wedge (Fig. 2b). The pattern of deformation changes only by increasing in extent with time, proportionally to the rate of indentation. For most problems, however, the actual shape of the boundaries, and, hence, the boundary conditions, change as deformation progresses. The plane indentation solution shown in Fig. 2a is an example; the slip lines are appropriate only for the onset of yielding.

In principle, it is possible to determine the finite deformation of a rigid-plastic medium by successively and incrementally calculating the deformation for the given boundary conditions and re-evaluating the boundary conditions on the deformed boundaries. For the analogy with Asian tectonics, however, we do not know with confidence the detailed history of deformation, and therefore sophisticated computation of complicated deformation histories probably will not be very useful at present. On the other hand, we think we do know, qualitatively, how deformation occurred during the past few million years, and we are more concerned with understanding it than speculating on the previous history of deformation. Fortunately, because the solutions for plasticity problems are incremental, in so far as strain hardening can be neglected, knowledge of the previous history of deformation is not necessary for calculation of the slip lines at a given stage of the deformation.

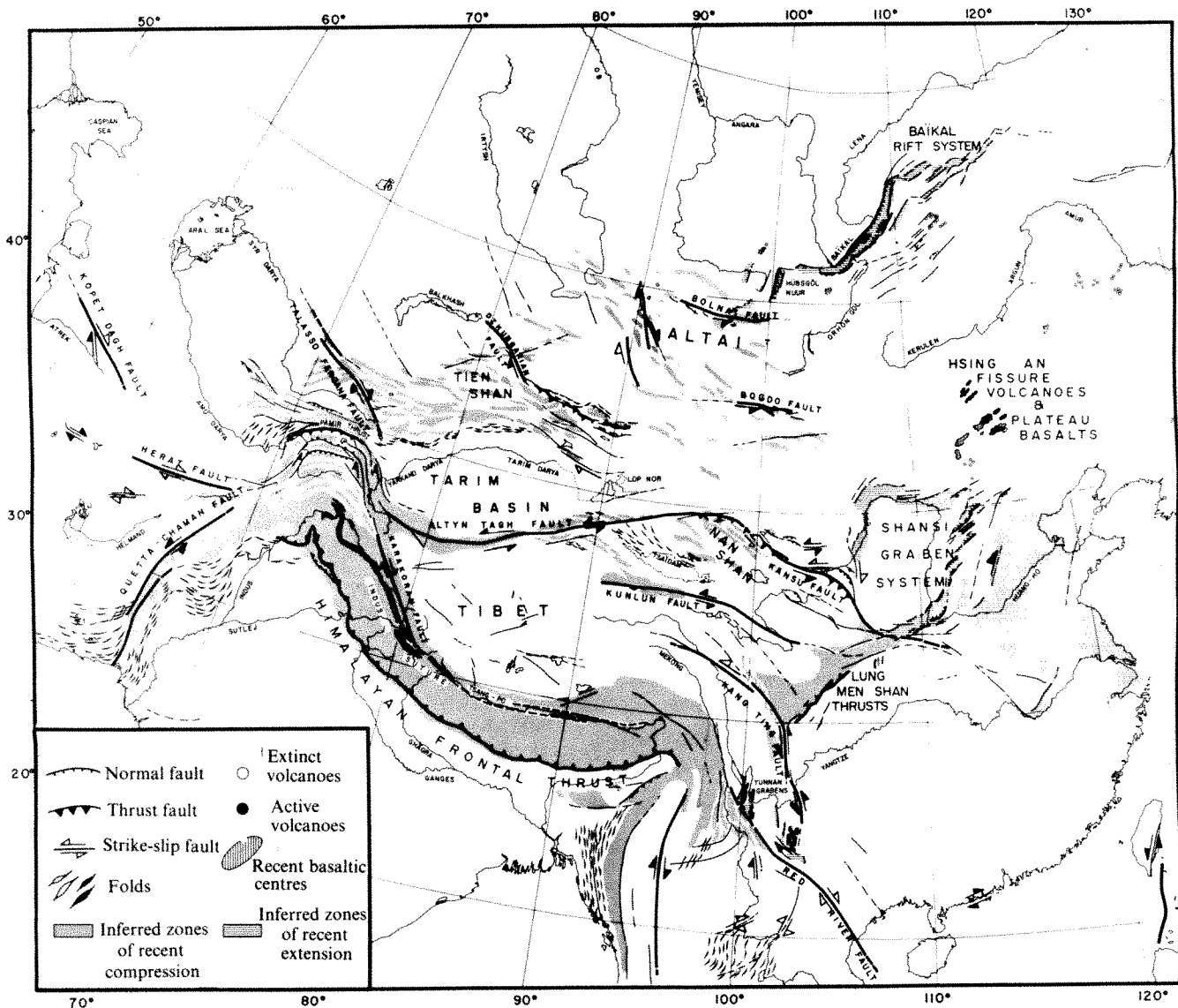


Fig. 1 Simplified map of recent tectonics in Asia⁵. (—) Major faults; (—) sense of motion; (▨) region of crustal thickening; (▨) regions of crustal thinning.

Assumption of plasticity and plane strain in Asian tectonics

Although in detail there are obvious violations of the basic assumptions made for the solutions for slip lines of rigidly indented rigid-plastic media when this formalism is applied to Asia, the similarity of the patterns alone is enough to pursue the analogy further. Although strain may accumulate elastically during intervals between earthquakes, the total strain in Asia since the collision of India is a few tens of per cent⁶. Elastic strain at any instant and the total change of volume in the region will be negligible portions of the total deformation.

If the stress on and near active faults alternately accumulates and then drops during earthquakes, the regional stress-strain curve may resemble that shown in Fig. 3b. Although the stress may vary with time, when averaged over a long time (many earthquakes), the regional stress-strain curve would be similar to that shown in Fig. 3a, for a perfectly rigid-plastic solid. In the Earth, at slow strain rates, strain hardening may be a minor effect, particularly along fault zones where gouge alteration and strain heating are likely to occur. Moreover, at depths ≥ 20 km fault creep is likely to play an increasingly important role in the deformation of the crust. At even greater depths, the temperature is appropriate for plastic deformation to be

dominant in most rocks at grain scale. Thus if most of the lithosphere at depth behaves plastically, in spite of the pressure dependent strength of the rocks in the top part of the crust, the assumptions of rigid plasticity and isovolumetric deformation are likely to be reasonable over a large scale and long time (10^5 yr) for the behaviour of the continental lithosphere as a whole.

Also, a plane horizontal strain analysis is justified for most of the deformation in Asia in the past 40×10^6 yr since the largest displacements (of the order of several hundred km (refs 5, 6)) seem to be horizontal movements along great vertical strike-slip faults.

Plane horizontal strain, however, does not take place everywhere in Asia. Important crustal thickening occurs at the Himalayas and Pamirs, and to a lesser degree in the Tien Shan and Nan Shan (Fig. 1). Further to the north-east (Fig. 1), crustal thinning occurs in the Baikal and Shansi graben belts. The deformation in these narrow mountain and graben belts, in fact, is close to plane vertical strain⁷⁻⁹, but is likely to be a fraction of that due to large scale strike-slip faulting, for two reasons: first, whereas strike-slip faulting is compatible with steady state deformation for long periods of time, in any given area crustal thinning is limited by the thickness of the crust itself and crustal thickening by the fact that, with increasing elevation

of the mountains, an increasing amount of work must be done against gravity. Second, the horizontal maximum principal stress necessary to cause plane strain ($\sigma_1 = \sigma_3 + 2\tau_Y$) is less for plane horizontal strain through strike-slip faulting where $\sigma_3 < \sigma_2 = \sigma_z$ than that for plane vertical strain through thrusting and crustal thickening where $\sigma_3 = \sigma_z$. Crustal thickening will occur when σ_1 is especially high as is the case in front of the indenter or where there are pre-existing weak fault zones of the right orientation, as might be the case in old orogenic belts such as the Tien Shan or the Nan Shan (Fig. 1). In fact, that the crust and upper mantle structure of Asia is not homogeneous and may not be isotropic even on a scale of a few tens of km may be one of the causes of the differences between the fault pattern in Asia and the slip-line fields.

Indentation geometry and Asian tectonics

The deformation of a rigid-plastic body caused by indentation depends strongly on the shape of the indenter. If the indenter is flat, deformation extends into the semi-infinite rigid-plastic medium to a distance approximately equal to the width of the

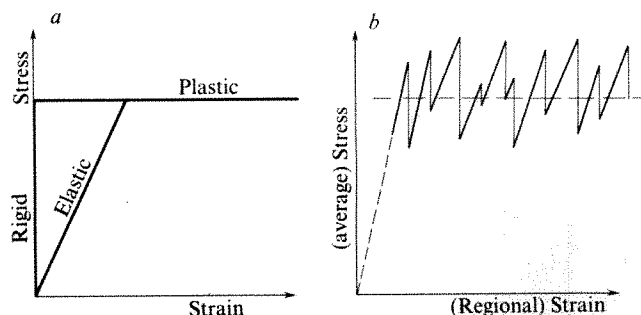


Fig. 3 *a*, Deviant stress-strain curve for rigid-plastic and elastic-plastic body. Once the yield stress, τ_Y , is reached, plastic strain proceeds at constant stress (no strain hardening). *b*, Qualitative regional stress-strain behaviour of rocks in top part of crust. Slow accumulation of elastic strain is followed by sudden stress drops during earthquakes. With increasing regional strain, average deviant stress may oscillate about roughly constant value.

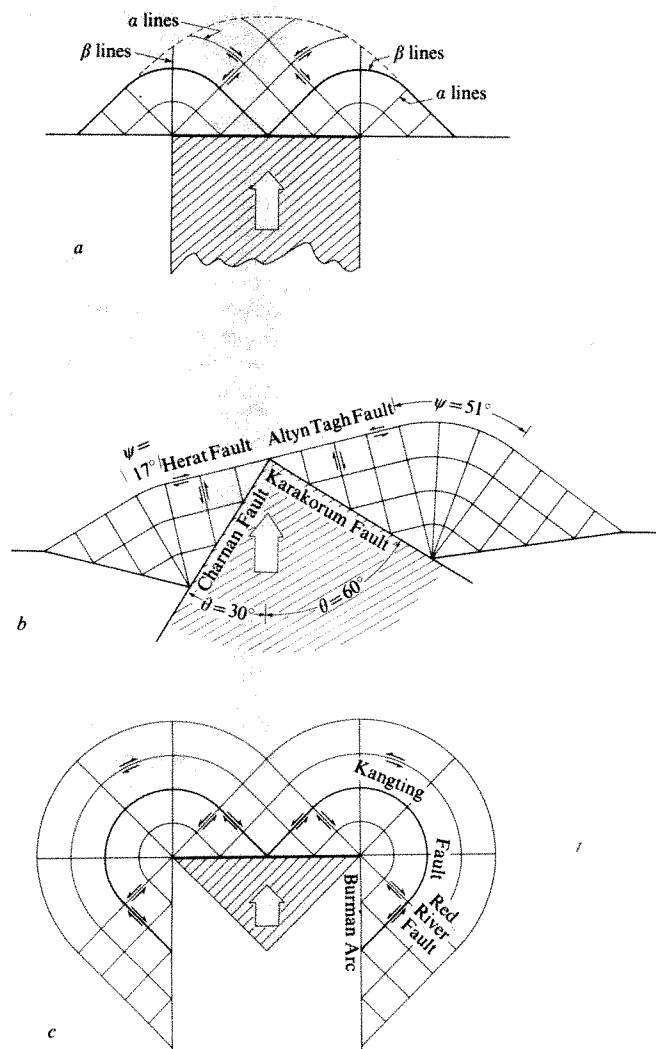


Fig. 2 Plane indentation of semi-infinite rigid-plastic media by different rigid dies. Arrows indicate sense of shear along slip lines. Principal stresses σ_1 and σ_3 bisect small quadrilaterals delineated by slip lines. The names of corresponding major tectonic features are indicated. *a*, Flat rigid die; *b*, rigid wedge—similar to the situation that occurs at the Pamirs (Fig. 1) (western end of the Himalayas); *c*, flat triangular indenter and hollowed-out medium, similar to situation which arises at the Himalaya-Burman syntaxis (Fig. 1) (eastern end of the Himalayas).

indenter (Fig. 2a). If the indenter is a wedge, at any given time the deformation may or may not extend further into the medium than the wedge itself depending upon the angle of the wedge (Fig. 2b).

Assuming the boundary of (rigid) India to be along the Himalayan Front and through the Kirthar and Sulaiman Ranges in Pakistan, parallel to the Chaman Fault, it seems that India has penetrated far into (plastic) Asia (Fig. 1), and therefore the analogy⁵ in Fig. 2a can be improved upon. In the western Himalayas and in Pakistan, the boundary of India is similar to that of a wedge. Note that the Herat and Altn Tagh faults are approximately parallel to the slip lines that would be created by wedge indentation (Fig. 2b). As the direction of motion between India and Eurasia is approximately north-south, the western edge of the Indian wedge is steeper than the eastern edge. Correspondingly, the Altn Tagh fault strikes more north-easterly than the Herat fault. The right lateral North Anatolian fault in Turkey⁴ and the left lateral Great Kabavi fault in Iran¹⁰ have an analogous relationship to Arabia, which appears to act as a wedge into Eurasia further west.

This simple analogy would predict strike-slip motion along the boundaries of the wedge and the rigid-plastic medium (Fig. 2b). Strike-slip motion of the right sense is observed for both the Chaman fault and the Dead Sea fault. There is also evidence for strike-slip faulting along the Zagros suture zone¹¹ and the Karakorum fault^{5,12}. In addition, however, thrust faulting and crustal thickening occur in both the Zagros and the Himalayas^{4,5,13}. The direction of thrust faulting is approximately parallel to the orientation of the maximum compressive stress in the plastic material (Fig. 2b).

An obvious feature of Asian tectonics is that deformation occurs over a vast area north-east of the Himalayas. If the wedge indentation analogy held for all of Asia, we might expect deformation north-east of the Himalayas to be bounded approximately by the Altn Tagh fault, just as deformation does not extend far north-west of the Herat fault. A possible explanation for the widespread deformation north-east of the Himalayas lies in the fact that the trend of the Himalayas to the east curves and becomes approximately perpendicular to the direction of relative motion of the plates. Thus the analogy with the indentation solutions in Fig. 2a would be more applicable to this region.

We think that the pattern of faulting north, east and even south-east of the eastern Himalayas supports the analogy with plane indentation by a flat indenter. The Kunlun and Kangting faults are approximately parallel to β lines. The marked curvature, particularly of the Kangting fault, compared with the straight Altn Tagh and Herat faults would be expected from their proximities to the edge of a flat indenter (Fig. 2a) and to a wedge (Fig. 2b) respectively.

For the region at the eastern end of the Himalayas an even

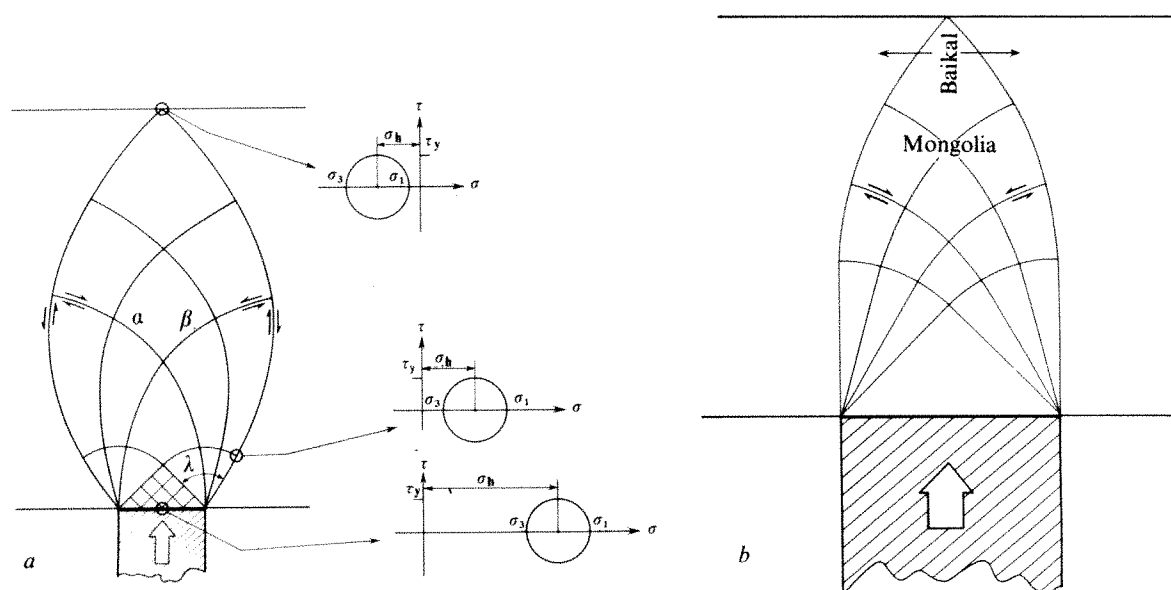


Fig. 4 Plane indentation of a bounded rigid-plastic medium². *a*, Width of plastic block is ~ 4.4 times width of indenter. Mohr circles on right show stress state at different points of field. σ_h decreases away from indenter as function of angle λ travelled along slip lines and becomes negative near boundary opposite indenter. *b*, Secondary tension may be cause of crustal extension at Baikal rift system.

more appropriate analogy than plane indentation of a flat-sided rigid-plastic medium is with the indentation of an already indented or a hollowed-out medium (Fig. 2c). For this case, the slip lines continue to curve around 180° in order to intersect the 'north-south' boundaries at 45° . In our interpretation of the Landsat photos, the Kangting fault curves around so that east of the Himalayas it trends north-south before becoming lost in the complications of the tectonics further to the south^{5,6}. In addition, the right lateral Red River fault^{5,6} is approximately parallel to an α line. Although this fault is longer than might be expected from the analogy in Fig. 2c, this analogy is strongly supported because it predicts not only the curvature and sense of motion on the Kangting fault but also the sense of motion and trend of the Red River fault.

For us a particularly puzzling feature was the Burman arc, in front of which are a series of folds and east-directed thrusts

and beneath which lies a belt of intermediate-depth earthquakes. Both observations imply a recent eastwards underthrusting beneath the arc. Thus, underthrusting of India beneath the Himalayas in a northerly direction and beneath the Burman arc to the east or south-east occurred simultaneously. In the indentation problem in Fig. 2c, material flows around the edge of the indenter and back in towards it. By analogy, we would expect India to squeeze part of Asia out of the way, so that this part of Asia extrudes around the edge of India and then encroaches upon its eastern side. As the Indian subcontinent is very narrow in eastern India between the Bay of Bengal and the Himalayas, oceanic lithosphere would have underthrust the Burman arc. Therefore resistance to the encroachment of extruded Asia would be less than if it encountered continental lithosphere. The Himalayan-Burman syntaxis and the surrounding tectonics are analogous to a corner in an indenter and the

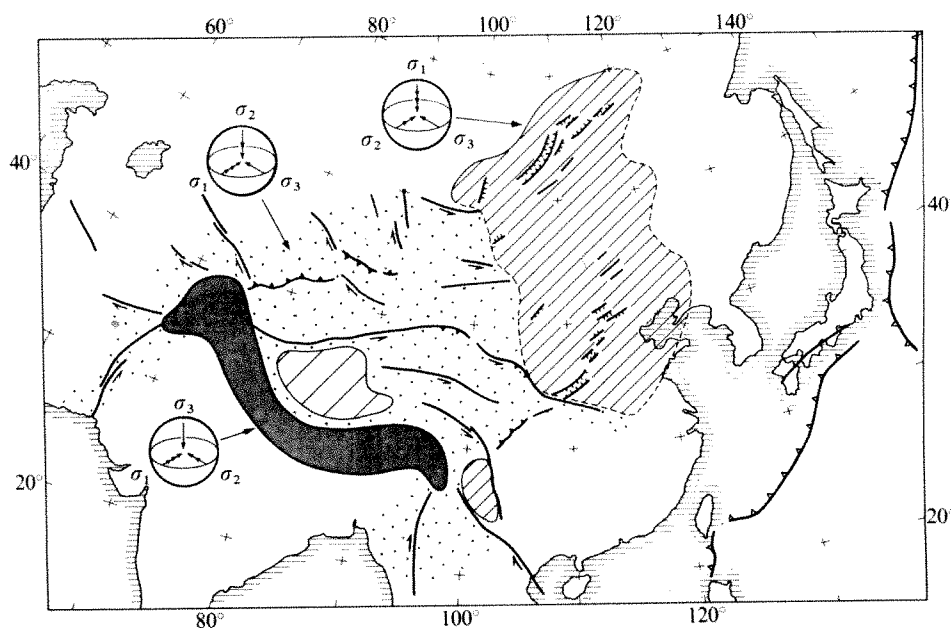


Fig. 5 Distribution of tectonic styles in Asia. Bold lines represent major faults (Fig. 1) (open thrust fault symbol for Pacific subduction zones). Dark shaded area—region of major crustal thickening, dotted area regions—where strike-slip faulting occurs, hatched area—normal faulting and crustal thinning. Corresponding stress states are indicated.

surrounding deformation when the indenter penetrates deeply into a rigid-plastic medium.

Boundary conditions at the limits of the indented block

The pattern of deformation in plane plastic strain also depends upon the shape of the boundaries, of the rigid-plastic medium, as is already clear from the differences in slip-line fields for the situations shown in Fig. 2a and c. For plane indentation as shown in Fig. 2, it is assumed that the rigid-plastic medium extends to very large distances compared with the width of the indenter. When a plastic block of finite width is indented, the slip-line field is of the type shown in Fig. 4a. Plastic yielding occurs in the region between the indenter and the symmetric pair of slip lines that leave the corners of the indenter and intersect each other at the opposite side of the block. Thus the plastic region becomes wider as the ratio of the width of the block (h) to the width of the indenter (a) increases (Fig. 4a). When this ratio reaches ~ 4.4 , the deformation changes drastically to resemble that in Fig. 2a; material flows around the edges of the indenter instead of forcing the two parts of the block to separate rigidly away from a narrow zone of plastic flow between them². Because the lithospheric plates are bounded, the slip-line field in Fig. 4a might be more appropriate for some problems than that in Fig. 2a. In a perfectly plastic material, the slip-line field will presumably be that either in Fig. 2a or in Fig. 4a, but not both, and not a combination of them. In the Earth, it is possible that elements of both situations might find analogues simultaneously, both because the continents are not homogeneous and because the shape of the plate boundaries and the boundary conditions are more complicated than in the idealised situations of Figs 2 and 4.

Perhaps the most interesting characteristic of slip-line fields of the type shown in Fig. 4a is that a tensile state of stress (secondary tension²) develops in the region adjacent to the boundary opposite the indenter. The cause of this tension is the

decrease of σ_h in the plastic medium away from the indenter. This decrease is expressed in Hencky's second theorem¹⁻³

$$\sigma_h = \sigma_{h(i)} - 2\tau_v\lambda$$

where $\sigma_{h(i)}$ is the maximum hydrostatic pressure in front of the indenter and λ the angle rotated clockwise along β lines and anticlockwise along α lines (Fig. 4). Along with σ_h both principal stresses σ_1 and σ_3 decrease proportionately to λ (Fig. 4a). In the extreme case shown in Fig. 4a, where $h/a \simeq 4.4$, σ_h is reduced enough near the apex of the field that all stress components are tensile. σ_3 becomes negative already in the middle of the field. This basic pattern is observed for all slip-line fields of the class shown in Fig. 4.

If the analogy of plane horizontal strain was strictly applicable to the Earth, then at a given depth the lithostatic stress σ_z would be equal to the intermediate stress $\sigma_2 = \sigma_h$. In the Earth, σ_z need not always be equal to σ_h , and when it is not, plane horizontal strain should not occur. There are two extreme situations in which this might occur: when $\sigma_z > \sigma_1 = \sigma_h + \tau_v$ or when $\sigma_z < \sigma_3 = \sigma_h - \tau_v$. These are likely to happen far from the indenter or close to it, respectively. Far from the indenter, the stress state will be appropriate for crustal thinning and the formation of graben (Figs. 4b and 5). We think that the Baikal Rift and Shansi grabens are a consequence of the stress system in Fig. 5, which is analogous to that in the area of secondary tension in Fig. 4. The predominantly normal faulting, the direction of extension and the relative position of the area in which it occurs to the Himalayas are appropriate to the conditions in Fig. 4b.

Deformation near the indenter, and mountain building

Near the indenter, σ_h is maximum and can be very large. When indentation starts it is likely that it will be large enough that σ_z will be the least compressive stress. Consequently, thrust faulting and crustal thickening will take place in the Earth. This in turn will lead to an increase in elevation and therefore to an increase in σ_z at a given depth below sea level. Plane horizontal strain will not take place until σ_z is large enough to be approximately the intermediate principal stress. At this stage, deformation may proceed through strike-slip faulting. This pattern of a first major phase of deformation with shallow thrusts and folds and a late phase of predominantly strike-slip faulting is the basic pattern to be found in most ancient orogenic belts⁸.

When plane horizontal strain is the dominant mode of deformation, then the mean elevation in any given area should reflect the local value of σ_h . The high altitude of Tibet and the general decrease in elevation away from Tibet in Asia may be a manifestation of the decrease in σ_h . Thus we think that both the distribution of tectonic styles in Asia (Fig. 5) and the decrease in mean elevation away from the Indian indenter may reflect a variation of σ_h similar to that predicted by the analogy in Fig. 4a.

The yield stress of Asia

For simple indentation geometries, the pressure σ_1 applied by the indenter and the yield stress are simply related. For the case in Fig. 2a, $\sigma_1 = 2\tau_v(1 + \pi/2)$; for that in Fig. 2, $\sigma_1 = 2\tau_v(1 + \pi)$; and for wedge indentation (Fig. 2b), $\sigma_1 = 2\tau_v(1 + \psi)$, where ψ is related to the wedge half angle θ by (see ref. 1)

$$\cos(2\theta - \psi) = \frac{\cos\psi}{1 + \sin\psi}$$

For $\theta = \pi/4$, $\psi \simeq 0.575$.

Assuming Tibet to be in isostatic equilibrium, we can estimate σ_1 for different depths of compensation. Because Tibet

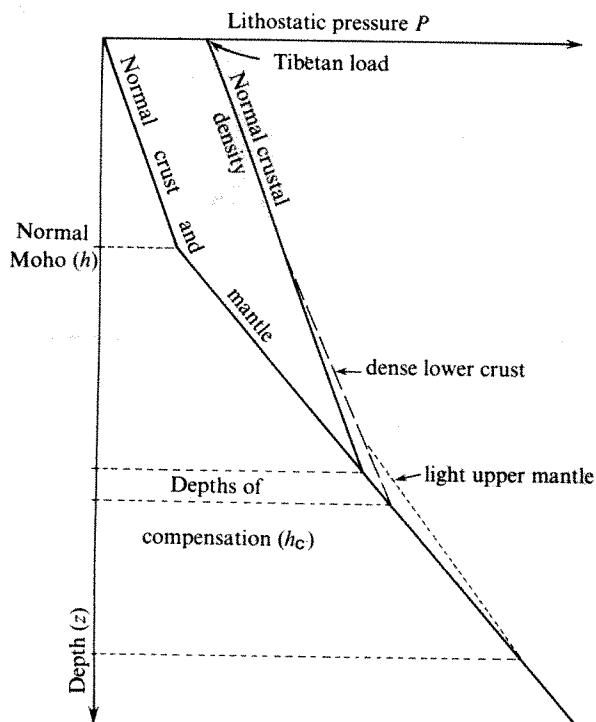


Fig. 6 Schematic plot of lithostatic pressure versus depth under both Tibet and normal crust. Down to normal Moho (h) the difference in lithostatic pressure is approximately constant and proportional to elevation difference $(1.2-1.4) \times 10^8$ Pa. Pressure difference then decreases to zero at depth of compensation h_c . Average value of pressure difference over thickness of layer above depth of compensation is $\sim \sigma_1$, the stress India applies to Eurasia in the plane indentation analogy.

is 5 km higher than the surrounding stable continental areas, for all depths shallower than the depth of compensation, the lithostatic pressure will be greater than that beneath the surrounding stable areas (Fig. 6). If we integrate this pressure difference from the surface to the depth of compensation, we obtain an estimate of the force per unit length, or the average pressure times depth of compensation, acting along the boundaries of Tibet^{14,15}. The value of this quantity depends upon how compensation occurs, but for all models in which compensation occurs primarily by crustal thickening beneath Tibet, the value is $\sim 5 \times 10^9$ Pa km⁻¹ (1 bar = 10^5 Pa).

By dividing this number by the thickness of the zone, H , assumed to behave analogously to a rigid-plastic material, we obtain an average estimate of σ_1 . For a 100-km thick zone, $\sigma_1 = 5 \times 10^7$ Pa. From the relationships above, the yield stress, τ_y , would be between $\sim 6 \times 10^6$ and 1.6×10^7 Pa. For a 50-km thick zone, these values should be doubled.

These estimates of σ_1 are only approximate and depend upon the assumption that the elevation of Tibet and the compensating mass deficiency are not maintained by vertical shear stress. Moreover, they represent average pressures, averaged over an assumed thickness (H), in which mechanical properties vary markedly with depth. If the yield stress estimated above was controlled essentially by the strength of the faults in the upper crust, where strain is relieved by earthquakes, the estimated strength of this brittle region would be larger than the values of τ_y given above.

For a range of thickness for brittle deformation of 20–40 km (ref. 16), the average stresses on faults would be 2.5–5 times larger than those estimated above, that is as small as 1.5×10^7 Pa or as large as 8×10^7 Pa. The number of assumptions make it impossible to be more precise. Moreover, stress could concentrate in localised regions on faults (asperities) to much higher levels, and the average stress may not reach 1.5×10^7 Pa for all earthquakes. In any case, the estimated range of average shear stresses on faults is higher than nearly all calculated stress drops of earthquakes (10^4 – 10^7 Pa), but is $< 10^8$ Pa.

Summary

When the pattern of faulting in Asia is compared with slip-line fields calculated for various plastic plane strain indentation problems several seemingly unrelated phenomena in Asia appear to have a common cause. India behaves like a rigid die that indents the rest of Eurasia, causing deformation over a large area that resembles deformation in rigid-plastic media. The linear Herat and Altyn Tagh faults are similar to α and β lines, respectively, caused by wedge indentation by north-western India. The more curved Kunlun and Kangting faults

are similar in sense and trend to slip lines near the edge of a more planar indenter, in eastern India. The right lateral Red River fault and the convergence at the Burman arc are parallel to α lines and the direction of plastic flow, respectively, for deformation near the corner of an indenter that has already penetrated deeply into a rigid-plastic solid. The simultaneous convergence at the Himalayas and flow around its eastern end causing convergence at the Burman arc is responsible for the Himalaya–Burman syntaxis. Tension is predicted at a large distance from a flat indenter, and the Baikal Rift Zone and Shansi Graben may be consequences of an analogous state of stress in Asia. From an estimate of the indentation pressure needed to maintain the high elevation of Tibet, we estimate that the average yield stress in a 100-km thick zone in Asia is $\sim 10^7$ Pa. If all of the yielding occurs by stress accumulation on faults and subsequent stress drops during earthquakes to a depth of 20–40 km, the average stress on the faults is a few hundred bars and less than a kbar.

We think that the analogy of plastic plane strain with the tectonic processes in Asia supports the contention that most of the tectonics of Asia are caused by the collision of India with Eurasia and provides a unifying explanation for the phenomena occurring there and in continental collisions in general. This type of approach may lead to an even more general understanding of both the geographical distribution of different tectonic styles within continents at given epochs and the succession in time of different tectonic phases in a given continental area.

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A complete skeleton of the Late Triassic ornithischian *Heterodontosaurus tucki*

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The first ornithischian dinosaur to be found in the Triassic (other than very fragmentary material) was Heterodontosaurus tucki from South Africa, described¹ on its skull. The postcranial skeleton of another H. Tucki is described here. Heterodontosaurus is compared with its sympatric contemporary Fabrosaurus, the only other well known Triassic ornithischian, and the interrelationships of early dinosaurs are discussed briefly.

LATE Triassic ornithischian dinosaurs fall into two distinct families: the Heterodontosauridae, including the genera *Heterodontosaurus*², *Lycorhinus*², *Abriotosaurus*² and *Lanasaurus*³, and the Fabrosauridae with the single genus *Fabrosaurus*⁴⁻⁶. (*Fabrosaurus*, however, is considered by some⁷ to be gen. indet.) The general structure of the skull in these two families is known in broad detail^{1,4,5,7-9}, and in *Fabrosaurus* the postcranial skeleton too is fairly well known⁶. Very little has been published hitherto, however, on the postcranial skeleton of *Heterodontosaurus*. This article is essentially a brief account of a virtually complete skeleton of *H. tucki*.

The specimen (South African Museum no. K1332) was discovered in December 1966 in the Upper Red Beds of the Stormberg Series, at a height of about 1,770 m above sea level on the northern slopes of Krommespruit Mountain in the District of Herschel, Republic of South Africa. More complete locality data will be published when the skull and skeleton are described in detail. The scapulo-coracoid, humerus and hand of the specimen have already been partly figured elsewhere¹⁰, though to a very small scale. The only other Triassic ornithischian known with postcranial material worth mentioning is the poorly preserved and fragmented *Pisanosaurus mertii* (ref. 11 and J. F. Bonaparte, unpublished) from the Ischigualasto Formation of Argentina.

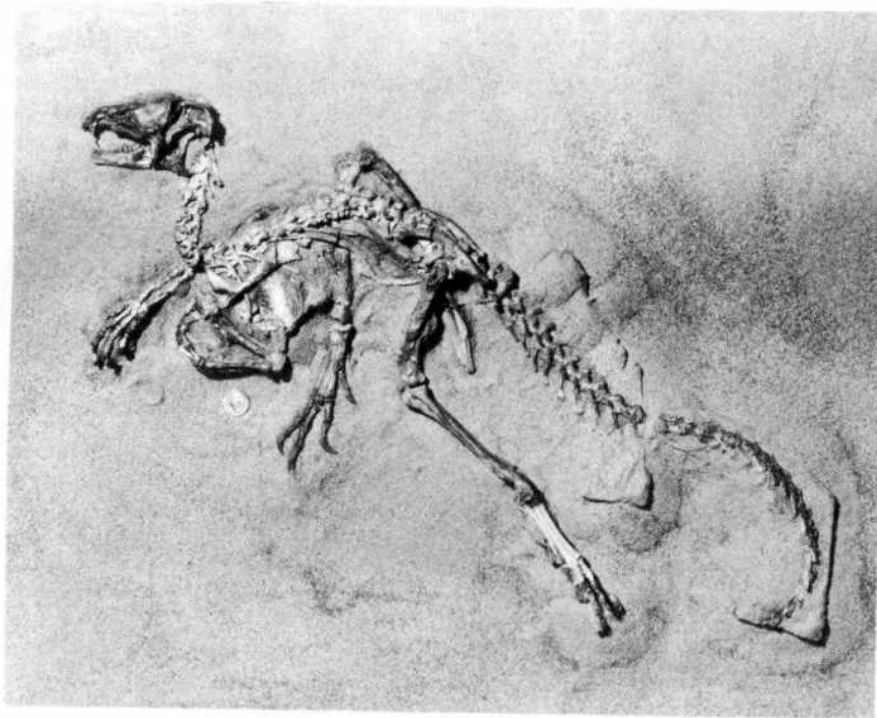


Fig. 1 *Heterodontosaurus tucki*. The specimen described in the text (SAM no. K1332).

The skeleton of *Heterodontosaurus* described here is preserved in an articulated condition with little displacement or distortion (Fig. 1). Figure 2 is the first ever complete reconstruction of the skeleton of a Triassic ornithischian. Measurements of some of the elements are given in Table 1.

Axial skeleton

The skull is almost identical with that of the holotype^{1,7,8}. The vertebral column includes 21 presacral vertebrae and 6 sacra, the latter fused together. (These figures, 21+6, compared with the typical thecodontian count of 25+2, suggest that the four additional vertebrae incorporated into the sacrum are all dorsals rather than caudals. The presacral count of 21 has not been broken down into separate cervical and dorsal counts because presacrals 8-10

are transitional in form and the exact position of the boundary between neck and trunk cannot be determined objectively; however, 9 cervicals plus 12 dorsals seems to be the most reasonable allocation.) Twenty-eight caudal vertebrae are preserved in two continuous series, nos 1-11 and (probably) nos 17-33, separated by a gap representing about five vertebrae. It is not possible to estimate accurately the number of vertebrae required to complete the column to the tip of the tail, but it could not have been many because the last vertebrae preserved are very small. Chevrons are present between and below most of the caudals. The remains of ossified tendons lie alongside the neural arches from the middle of the trunk to the posterior end of the sacral series, but they are absent from the tail.

Pectoral girdle and forelimb

The scapula and coracoid are slightly curved, with the concave surface medially; the curve of the coracoid is a little more pronounced than that of the scapula. The dorsal region of the scapula forms a wide blade and appears to have been capped by a cartilaginous extension, while below the blade the scapula narrows to a nearly circular cross section. The wider ventral region supports a well developed acromial process on its anterior margin. A powerful tubercle, presumably for the origin of the scapular head of the triceps muscle, is situated immediately

above the dorsal lip of the glenoid. Near the forelimb lies a roughly rectangular sternal plate with a weakly concave anterior edge, a convex medial edge and straight lateral and posterior edges.

The humerus is slightly twisted along its longitudinal axis; its head is distinct, and the strongly developed deltopectoral crest is so long that it occupies about 40% of the length of the whole bone. There is no olecranon fossa on the posterior surface, in spite of the presence of a distinct olecranon process on the ulna; the anterior surface of the humerus, however, bears a shallow depression above the condyle. The entepicondyle is large, suggesting that the flexor muscles for the forearm and manus were strong. The radius too is slightly twisted about its longitudinal axis, and a small crest, 10 mm high, arises from the middle of the lateral surface of the shaft. The carpus consists of nine

bones: a radiale, an ulnare, a pisiform, a centrale and five distal carpals. The ulnare, with a highly concave proximal surface and a nearly flat distal surface, is the largest of these; a small foramen pierces its dorsal surface. The radiale has the same transverse width as the ulnare but is flatter. Of the distal carpals only V shows reduction, in spite of the fact that digits IV and V are both reduced; indeed, distal carpal V is actually smaller than the pisiform.

The manus is extremely long, the length of digit III being nearly 52% of that of the humerus. The phalangeal formula is 2-3-4-3-2. The distal articular surface of each of the metacarpals I-III is extended dorsally and proximally on to the dorsal surface to an unusual degree; this suggests that the proximal phalanx of the corresponding digit could have been hyperextended on the metacarpal. The distal end of metacarpal I is twisted relative to its proximal end, likewise the distal end of the proximal phalanx of both digits II and III; this would have caused digits I-III to move mediad during flexion, thus producing an effective grasping motion of the manus. Further, the terminal claw-bearing phalanges (unguals) on those three digits bear pronounced flexor tubercles.

Pelvic girdle and hindlimb

The ilium has a long, shallow anterior process which terminates in a small knob-like expansion. The posterior process is slightly shorter and slightly deeper and its posterior

marks the attachment of the ischio-trochanteric and internal tibial flexor muscles¹⁴.

The femur is relatively short and slightly curved with a convex anterior surface; the head projects nearly at right angles to the longitudinal axis of the shaft, the greater trochanter is not separated from the lesser trochanter by a marked cleft, and a pendent fourth trochanter, about 10 mm long, forms an angle of approximately 45° with the axis of the shaft. The tibia is very long, 1.29 times the length of the femur; its proximal articulating surface is not horizontal but slopes downwards and outwards from its medial edge. The proximal part of the tibia is considerably expanded anteriorly into a strong cnemial crest. The head of the fibula also is expanded antero-posteriorly but, even so, its length is only half that of the tibial head; distally the fibula narrows to a thin rod which fuses with the tibia. The astragalus and the calcaneum are indistinguishably fused with the tibia and fibula. The three distal tarsals are fused with the metatarsal heads; thus the lateral distal tarsal caps metatarsal IV, the central caps III, and the medial distal tarsal covers both II and I.

The first four metatarsals are fused to form a single unit, their proportional lengths (taking the sum of their lengths as 100%) being 16.8%, 26.1%, 30.0% and 27.1% respectively. Metatarsal V, however, is reduced to a slender splint. The phalangeal formula is 2-3-4-5-0. Digit III continues the longitudinal axis of its metatarsal, but digit II

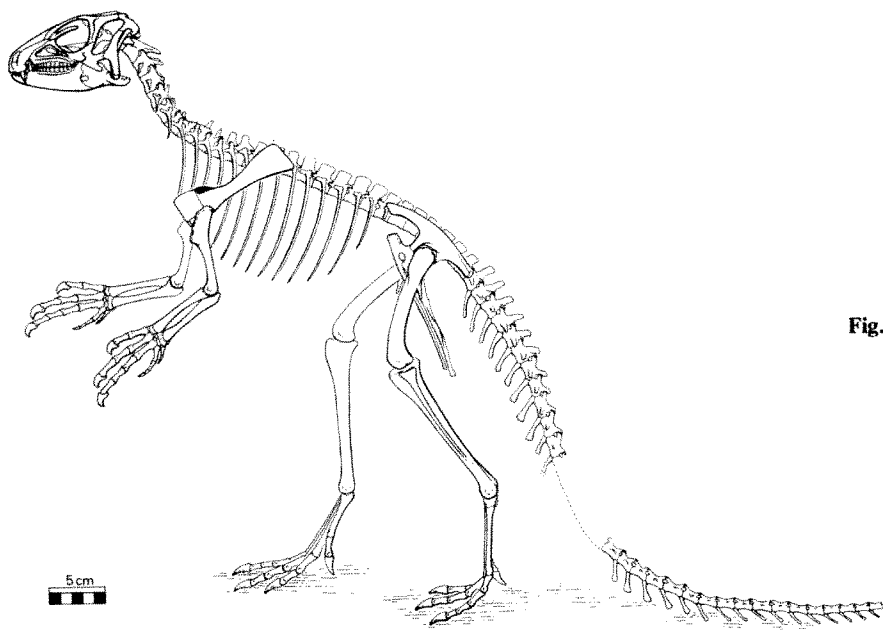


Fig. 2 *Heterodontosaurus tucki*. Reconstruction of the complete skeleton.

end also is a little expanded. The pubic peduncle lies almost at right angles to the longitudinal axis of the ilium. A mound-like protuberance and an articular facet on the lateral surface of the ischiadic peduncle increase the depth of the acetabulum. The pubis has a short, deep anterior ramus (15 mm long) and a long, straight, rod-like posterior ramus (pubis proper) which is nearly as long as the ischiadic rod lying immediately above it. Several small rounded tubercles are present on the lateral surface of the anterior ramus; these may be associated with the origins of the ambiens muscle and of the three heads of the pubo-ischio-femoralis internus¹²⁻¹⁴. The ischium is joined to the ilium by a short rounded process, but the process which attaches it to the pubis is flat, wide and expanded. Posterior to this pubic process the ischium narrows to a straight slender rod with a slightly expanded end; there is no obturator process on the ischium, but a prominent and gently curved crest on the central part of the rod probably

diverges medially and IV laterally; digit I is partly reduced and apparently had no role in support during locomotion. The unguals are slightly curved.

Discussion

Heterodontosaurus tucki seems to have been a bipedal cursorial herbivore; this opinion is based mainly on the structure of the manus and pes, the length ratio of tibia to femur, and the structure of the teeth and jaws. Although the forelimb may have been used for locomotion when the animal was moving slowly, for example when foraging, it seems to have been adapted primarily for grasping and tearing. A large olecranon process is usually present in quadrupeds like *Stegosaurus* but absent in bipeds such as *Fabrosaurus*; its presence in *Heterodontosaurus tucki*, however, may have been functionally associated with the powerful arm and highly manipulative hand, for the

Triassic coelurosaur *Syntarsus*¹⁸ also has both an olecranon process and a grasping hand.

The structure of the postcranial skeleton supports the view, based originally upon the skull structure, that the two families of Late Triassic ornithischian dinosaurs (namely the *Fabrosauridae* and the *Heterodontosauridae*) differ from one another in many important features. (This, in turn, accords with the belief that each family had a long independent history dating back to at least mid-Triassic times.) *Fabrosaurus* too was a cursorial biped, sympatric with *Heterodontosaurus*, but it lacks the deep skull of *Heterodontosaurus* and its powerful and specialised masticatory apparatus. Only five sacral vertebrae are present in *Fabrosaurus* instead of six. The deltopectoral crest and whole forelimb are relatively shorter in *Fabrosaurus* than in *Heterodontosaurus*, and *Fabrosaurus* does not have a long and powerful grasping hand. Further, the ulna of

The combination of specialised features seen in *Heterodontosaurus* seems to rule it out as a possible ancestor of known ornithopod ornithischians of later times¹⁸. These features include the nature of the dentition (the closely set teeth worn down to a continuous occlusal surface), the low number of dorsal vertebrae, the large grasping hand, the absence of the obturator process on the ischium, the reduced fibula and the complete fusion of the distal ends of the tibia and fibula with each other and with the proximal tarsals. Although so early in time, *Heterodontosaurus* is far more specialised in these and other characters than are the majority of much later ornithopods—such as *Hypsilophodon* and *Iguanodon* of the Early Cretaceous and even *Thescelosaurus* of the Late Cretaceous. Possible relationships can be discussed in detail only when the available material of Triassic dinosaurs has been more fully prepared and studied.

Bakker and Galton¹⁸ have stated that all Triassic dinosaurs share several common features, such as the shape and direction of the glenoid cavity, the position of the deltopectoral crest, the structure of the hand, the perforation of the acetabulum, the structure of the femur (in particular the position and size of the fourth trochanter), the restriction of femoral movements to the vertical plane, and the mesotarsal type of ankle joint. On the basis of these similarities they have concluded that the three major groups of Triassic dinosaurs (prosauropods, theropods and ornithischians—the last in effect restricted to ornithopods) arose from a central stock of early forms such as *Staurikosaurus*¹⁷, *Ischisaurus*¹⁸ and *Herrerasaurus*^{18,19}. This stock, according to them, could have been derived in turn from a group of thecodontians of which *Lagosuchus*²⁰ is a representative. Such a conclusion, however, seems hardly to accord with the view that even the Triassic ornithischians alone comprise two distinct families with long independent histories, probably going back to no later than mid-Triassic times. A fundamental distinction between the three main groups of Triassic dinosaurs lies in the structure of the pelvic girdle; the form of the prosauropod pelvis does not differ greatly from the primitive thecodontian pattern, whereas the theropod and ornithischian pelves are more highly modified and are also very different from each other. Bonaparte²⁰ is not totally in agreement with Bakker and Galton¹⁸ and still believes (following Charig^{21,22}, Charig *et al.*²³) that both saurischian suborders, as well as the ornithischians, were derived independently from thecodontians. It may well be that some of the common characteristics of Triassic dinosaurs as listed by Bakker and Galton were present in an ancestral thecodontian stock and that others were the result, at least in part, of parallel evolution. Some features of the hand of *Heterodontosaurus* seem to support this view; for example, the facts that metacarpals IV and V of *Heterodontosaurus* are divergent and that digit I does not diverge markedly from digit II are more reminiscent of the hands of thecodontians than of those of early saurischians. Likewise, one of us argues²⁴ that most of the alleged similarities between Saurischia and Ornithischia do not exist or are without phylogenetic significance; the lack of common characters 'primitive' to the supposed taxon Dinosauria, together with the existence of several important differences (not mentioned by Bakker and Galton) between the two orders, suggests that the alleged monophyly of the "Dinosauria" cannot be demonstrated.

It seems undeniable that there was a unity to dinosaur biology which greatly exceeded a few osteological features, but this may well have been due to the fact that all the groups concerned were in any case fairly closely related and had been subjected to the same evolutionary pressures. Whatever view is taken of the controversy concerning the origins and interrelationships of the major groups of dinosaurs, all will agree that it calls for the thorough and detailed preparation and description of the now extensive

Table 1 Selected measurements* of *Heterodontosaurus tucki* (SAM no K1332)

	(mm)
Scapula, maximum length	86.9
Coracoid, maximum length	22.3 estimated
Humerus, maximum length	82.3
Radius, maximum length	58.5 (right)
Ulna, maximum length	67.6
Metacarpals, maximum lengths: I	17.6
II	23.3 (right)
III	22.4
IV	14.5 (right)
Ilium, maximum length	96.9
minimum height above acetabulum	15.0
Pubis, maximum length	125.7 (right)
height of anterior ramus	7.8 (right)
Ischium, maximum length	114.2 (right)
Femur, maximum length	112.2
length of fourth trochanter	9.8
Tibia, maximum length	145.0 (right)
Metatarsals, maximum lengths: I	38.1 (right)
II	59.1 (right)
III	67.9 (right)
IV	61.4 (right)
Combined length of centra of twelve dorsal vertebrae	171.7
Hindlimb (femur + tibia + metatarsal III)	1.89
Trunk (12 dorsal centra)	

* Left side unless stated otherwise

Fabrosaurus lacks the olecranon process and its humerus lacks the prominent entepicondyle. The posterior process of the ilium is shorter and deeper, the pubic peduncle is directed forwards and downwards relative to the long axis of the iliac blade (rather than nearly at right angles thereto as in *Heterodontosaurus*) and there is a large obturator process on the ischium. There are also many differences between the two genera in the structure of the hindlimb. In *Fabrosaurus* the greater trochanter is separated from the lesser trochanter by a deep cleft, the fibula has an expanded distal end and the astragalus and calcaneum are fused neither with the tibia and fibula nor with each other. There is, however, one important feature of the pelvis common to both *Fabrosaurus* and *Heterodontosaurus*—a complete and undescribed pubis of *Fabrosaurus* (SAM no. K1106) shows that the anterior ramus is very similar in shape and even in size to that of *Heterodontosaurus*.

The skeletal remains of *Pisanosaurus*²¹ are very incomplete, but the lower jaw and dentition suggest a closer affinity with *Heterodontosaurus* than with *Fabrosaurus* (Ref. 7 and J. Bonaparte, unpublished). The separate fibula of *Pisanosaurus* with its unreduced distal end and the astragalus and calcaneum fused neither with the tibia and fibula nor with each other might be thought to suggest a relationship to *Fabrosaurus* closer than to *Heterodontosaurus*, but in fact such features are merely primitive and without phylogenetic significance.

collections of Triassic ornithischian dinosaurs and a comparison of these with all available material of their saurischian contemporaries.

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Cell length, cell growth and cell division

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When cells of E. coli reach a certain critical length, which is constant in all growth conditions and equal to twice the minimum cell length, they abruptly increase their rate of elongation and divide about 20 min later. Chromosome replication terminates at about this same cell length but is not the signal for the change in rate of cell elongation.

DURING exponential growth, cells of *Escherichia coli* divide after each doubling in their volume. Their growth rate, and thus the frequency at which they divide, depends on the temperature and the availability of nutrients in the culture medium. *E. coli* cells are cylinders with hemispherical ends and, at any one growth rate, the doubling in cell volume between successive division takes place entirely by a doubling in cell length, without detectable change in cell diameter¹. But at each growth rate one observes a different cell volume, length and diameter. The relationship between cell volume and growth rate has been known for some time² and explanations for this relationship have been given^{3–5}. Nevertheless there has not, to our knowledge, been a comparable systematic investigation of the relationship between growth rate and cell length in *E. coli* (although there are several scattered measurements in the literature). We have therefore carried out such an investigation and have found a simple relationship between cell length, cell growth and cell division. This is that individual cells become committed to division and increase their rate of elongation at a fixed length which is independent of growth rate. Moreover, the change in rate of elongation seems to depend solely on the attainment of the critical length and not on other factors such as the termination of chromosome replication (as has been suggested by others^{6–8}). Following a change in growth conditions, cells do not alter their rate of elongation so that it is proportional to their new rate of volume and mass increase, until they attain the critical length. As a consequence, cells change their size and proportions to those characteristic of the new growth rate within one division cycle after the change in growth conditions.

These experimental results are discussed in terms of a modified "unit cell" model⁹ in which the basic unit of cell growth is a section of cell of fixed length, Λ , which extends in length at a fixed number of growth sites (possibly one). The rate of cell elongation therefore doubles after completion of a new unit cell, at length 2Λ . The completion of a new unit cell occurs at the same time as does a signal for the commencement of septation. Septation takes approximately 20 min to complete in all growth conditions.

Relationship between cell length and growth rate

We have calculated mean cell lengths from cell length distributions of *E. coli* B/r (ATCC 12407) growing exponentially at

37 °C in a number of different media (Table 1). A linear regression line was calculated using the method of least squares, which gave

$$\bar{L} = (0.67R + 2.01) \mu\text{m} \quad (1)$$

(where \bar{L} is mean cell length in μm and R is the number of doublings in cell number h^{-1}). The correlation coefficient between the data and the regression line was $r = 0.96$ (Fig. 1).

Average cell lengths were also calculated for a K12 strain, OV2 (Table 1) growing in different media at three different temperatures. These points are also shown in Fig. 1, from which it can be seen that they obey the same relationship as the unrelated B/r. (For these points, $\bar{L} = 0.6R + 2.03$; for which $r = 0.88$.)

The relationship between mean cell length and growth rate shown in Fig. 1 has the following implications: (1) Cell length at a time approximately 20 min before division is approximately the same at all growth rates. (2) This length is approximately twice the minimum length of a cell in any growth condition.

We reach these conclusions as follows. For this argument we rewrite equation (1), without significant error, as

$$\bar{L} = (2 + 2R/3) \mu\text{m} \quad (2)$$

The length of cells at birth (L_B) and at division (L_D) may be calculated, to a first approximation, as proportional to cell age, using the theoretical distribution of cell ages in an exponential population^{1,10}, so that

$$L_B = \bar{L} \ln 2 \quad (3)$$

$$\text{and } L_D = 2L_B \quad (4)$$

(Mathematically more sophisticated methods of estimating these lengths give very similar results when applied to our population; see ref. 12.) Equations (2), (3) and (4) therefore allow us to calculate the lengths of cells at division for all growth rates. But to calculate cell lengths at different stages in the cell cycle, we must make some assumption as to how the cells increase in length (for example, at a constant rate or at a rate which increases as the cell cycle progresses). It is known that cells of *E. coli* increase in length continually over the cell cycle^{1,9,13} but the exact shape of the curve of increase is difficult to determine experimentally, both because of the smallness of the cells and because the change in rate of elongation is only twofold. Nevertheless it can be assumed that this curve lies somewhere between the extreme of a straight line (that is, a constant rate of elongation throughout the cycle) and a linear function in which the rate doubles some time during the cycle.

(An exponential rate of elongation would lie between these two curves.) In the special case in which the rate of increase is linear with the doubling 20 min before division, equations (2), (3) and (4) show that cell length at this time will be the same for all growth rates (Fig. 2b) and that this length is

$$(4 \ln 2) \mu\text{m} = 2.77 \mu\text{m}$$

Even in the cases where length increases exponentially (Fig. 2a) or at a constant rate over the whole cycle, these equations show that cell length 20 min before division will be approximately the same at all growth rates. These lengths will be $2.86 \mu\text{m} \pm 3\%$ and $2.96 \mu\text{m} \pm 6\%$ respectively. Thus cell length must be approximately $2.8 \mu\text{m}$ at a point 20 min before division at all growth rates.

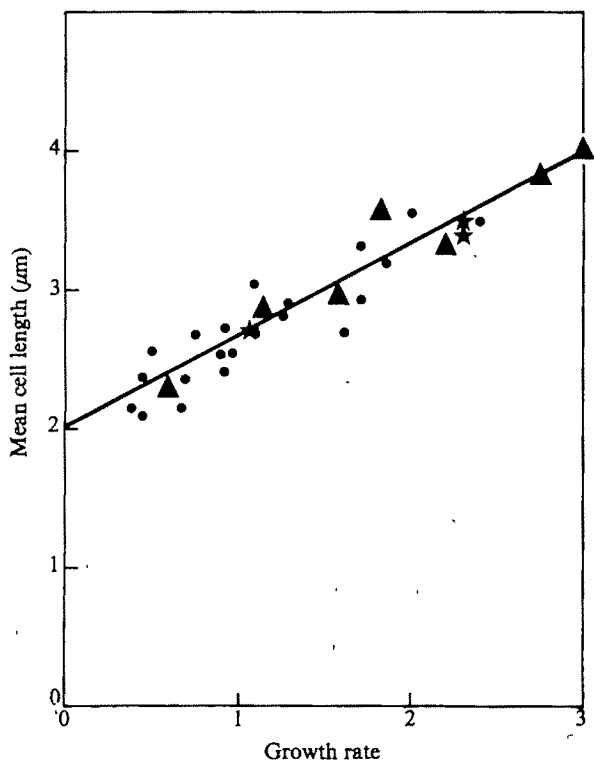


Fig. 1 Mean cell length of exponential phase populations of *E. coli* (data from Table 1) as a function of growth rate (doublings in cell number per hour). The line is the best-fit linear regression calculated by the method of least squares. (Triangles, B/r ATCC 12407; circles, OV2; measurements by light microscopy. Stars, OV2; measurements by electron microscopy).

The minimum length of cells in any steadily growing population may be estimated as the birth length at zero growth rate, using equations (2) and (3). The calculated value for this theoretical minimum length, Λ , is

$$\Lambda = 2 \ln 2 \mu\text{m} = 1.39 \mu\text{m} \quad (5)$$

Thus cell length 20 min before division must always be approximately twice the theoretical minimum cell length. In the special case shown in Fig. 2b, this length must be precisely 2Λ at all growth rates.

In the various models for cell elongation discussed above, it has been assumed that the rate of elongation will be a function of the growth rate in mass and number. This assumption works well for steady growth in a constant environment but it can easily be seen that it cannot apply without modification to cell growth during the transition between one growth rate and another. Thus, taking the model shown in Fig. 2b as an example, if the rate of elongation of a cell were to change immediately to that characteristic of the new environment after a shift from one growth medium to, say, a richer one, cell length at the time of

the next division would be greater than that actually found in the new medium. In fact, if the rate of elongation did change immediately after such a shift, cells would possibly maintain a constant diameter at all growth rates. This is not so. One possible solution to this paradox would be if the rate of cell extension remained the same after a shift to a new mass doubling time and changed to that characteristic of the new growth rate only after the cells reached a length of 2Λ . Our observations support this prediction and, in so doing, also provide evidence that the rate of elongation does change abruptly at about the time and cell length predicted by the model shown in Fig. 2b.

Figure 3a shows such an experiment. At zero time, a selected fraction of small cells was transferred from a poor growth medium (generation time 67 min) to a rich medium (generation time 25 min). The theoretical time of first division after such a transfer is about 60 min (ref. 14) and cells in fact divided between 40 and 60 min (Fig. 3a). The rate of mass increase of the cells increased immediately to the new rate¹⁵ but the rate of elongation remained at the calculated pre-shift rate until it increased abruptly by 5.7-fold after about 45 min. (Figure 3b shows a control experiment in which the selected cells were maintained in the same poor medium as before selection. In this case, the point at which the rate of elongation increased could not be determined, as expected, but the increase in rate over the first division cycle was close to twofold.)

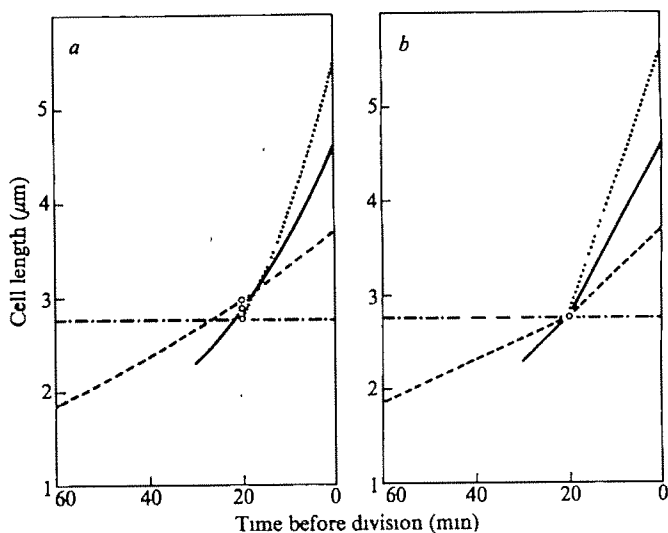


Fig. 2 Calculated course of increase in cell length over cell cycles of various durations, assuming that length doubles over the course of each cycle, that the relationship between mean population cell length and growth rate is given by the straight-line function (equation (2), Fig. 1), and that populations have the theoretical age distribution¹⁶ (see text). The figure shows cell elongation according to two sets of assumptions: a, that the rate of elongation increases continuously according to a logarithmic function, and b, that the rate of elongation is constant but doubles 20 min before division. For clarity, only four growth rates are shown, namely those for generation times of 20 min (· · ·), 30 min (---), 60 min (—) and infinity (— · —). The open circles represent cell length at 20 min before division.

The model we propose to describe cell elongation is therefore that newborn cells elongate at a constant rate which is proportional to the rate of growth in mass. Such newborn cells will always be between Λ and 2Λ in length and will reach 2Λ after a period of growth which is proportional to the initial cell length and the growth rate. In steady growth conditions, the rate of elongation will double at this point. (During a medium shift, this rate increase will be a doubling times the change in growth rate in mass.)

(The possibility that the sucrose gradient selection might perturb the rate of cell elongation may be discounted, since we observe the change in rate of cell elongation at the same cell

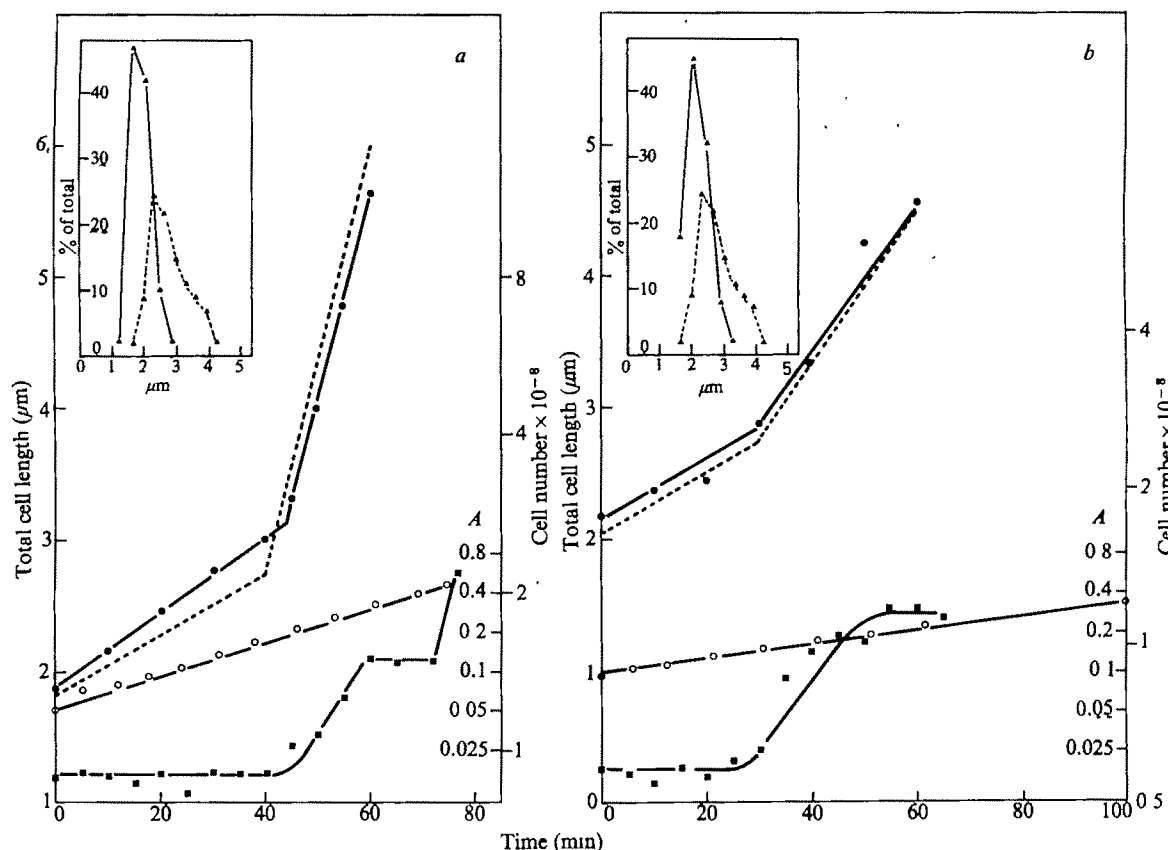


Fig. 3 Course of increase of total cell length (●), total cell mass (○) and total cell number (■) in synchronous cultures of *E. coli* B/r ATCC 12407. (Total cell length is calculated as mean cell length \times cell number per cell number at time zero. The error of estimate of mean cell length is $\pm 3\%$). In *a*, small cells were selected by sucrose gradient centrifugation²⁴ from a population growing exponentially in medium 2 (Table 1) with a generation time of 67 min and reinoculated into L-broth + glucose, after which the mass doubling time immediately decreased to 25 min. Cell division took place between about 40 and 60 min. Cell length increased at a constant rate for approximately 40 min and then increased abruptly to a new rate which was about $5.7 \times$ the initial rate. In consequence, the cell length and cell mass changed from those for cells growing at a rate of 0.9 doublings h^{-1} to those for cells growing at 2.4 doublings h^{-1} by the time of the first division in the new medium. The predicted course of cell elongation according to the theory developed in this paper is also shown (— — —). *b*, The result of a control experiment in which small cells from an exponential population with a generation time of 63 min (medium 2) were reinoculated into a fresh batch of the same medium. The selected fraction of cells were slightly larger than those obtained in *a* and there was a small initial stimulation of the rate of mass increase. The theoretical curve for length increase was drawn taking these factors into account. In this case the increase in rate took place at approximately 30 min and was an increase of about $2.3 \times$, as predicted. The insets show the length distributions of the selected populations at zero time, superimposed on the distribution for exponential asynchronous populations in the same growth conditions.

Table 1 Growth rate and cell length

Strain	Medium	<i>T</i>	37 °C <i>R</i>	\bar{L}	<i>T</i>	30 °C <i>R</i>	\bar{L}	<i>T</i>	42 °C <i>R</i>	\bar{L}
B/r ATCC	1	100	0.60	2.29						
B/r ATCC	2	52.5	1.14	2.88						
B/r ATCC	3	38	1.58	2.96						
B/r ATCC	4	33	1.82	3.54						
B/r ATCC	6	27	2.22	3.03						
B/r ATCC	7	22	2.73	3.83						
B/r ATCC	9	20	3.00	3.99						
OV2	1	67	0.90	2.54	135	0.44	2.09	135	0.44	2.37
OV2	2	65	0.92	2.73	160	0.38	2.13	120	0.50	2.57
OV2	3	65	0.92	2.41	87	0.69	2.36	90	0.67	2.15
OV2	4	47.5	1.26	2.82	80	0.75	2.71	62.5	0.96	2.55
OV2	5	35	1.71	2.93	57	1.08	2.70	—	—	—
OV2	6	—	—	—	—	—	—	37	1.62	2.68
OV2	7	35	1.71	3.32	55	1.09	3.06	32.5	1.85	3.19
OV2	8	30	2.00	3.56	47	1.28	2.89	25	2.40	3.49

OV2 is a spontaneous *ilv⁻his⁻* derivative of *E. coli* K12 M6K (see Table 2). *T* is the cell number doubling time in minutes, *R* is the number of doublings per hour, and \bar{L} is the mean cell length of the population in μm . Media: (1) M9+sodium succinate (0.4%); (2) M9+glycerol (0.8%); (3) M9+glucose (0.4%); (4) M9+glucose (0.4%)+histidine (20 $\mu\text{g ml}^{-1}$)+methionine (20 $\mu\text{g ml}^{-1}$); (5) M9+glucose (0.4%)+casamino acids (0.5%); (6) M9+glucose (0.4%)+casamino acids (0.5%)+tryptophan (20 $\mu\text{g ml}^{-1}$); (7) M9+glucose (0.4%)+casamino acids (0.5%)+tryptophan (20 $\mu\text{g ml}^{-1}$)+cytosine (20 $\mu\text{g ml}^{-1}$)+adenine (20 $\mu\text{g ml}^{-1}$)+uracil (20 $\mu\text{g ml}^{-1}$); (8) Oxoid Nutrient Broth no. 2; (9) Oxoid Nutrient Broth no. 2+glucose (0.4%). All OV2 cultures contained in addition all required auxotrophic supplements (final concentration of thymine was 50 $\mu\text{g ml}^{-1}$). For these measurements, cells were grown in log phase with shaking in liquid medium for several generations and samples taken at a density of 10^8 ml^{-1} (media 1–7) or $3 \times 10^7 \text{ ml}^{-1}$ (media 8 and 9) on to slides coated with a thin layer of agar[®] containing 0.1% sodium azide to prevent further cell growth. Cells were photographed with a Zeiss Ultraphot microscope under phase-contrast optics. Enlarged projections of the negatives were measured⁹. At least 200 cells were measured for each distribution. The error of estimate of \bar{L} was not more than 3%.

length in single cells growing in agar following a shift from a poor to a rich medium.)

Control of rate of cell elongation

We now ask what event in the cell cycle is responsible for the observed increase in rate of elongation which takes place about 20 min before division. It is known that the cell becomes committed to divide (even in the absence of further RNA, protein or DNA synthesis) at approximately 20 min before division¹⁶⁻¹⁸. This transition point in the *E. coli* cell cycle coincides with (1) termination of the oldest round of chromosome replication¹⁹, (2) segregation of the nuclear bodies¹⁴, (3) completion of the period of protein synthesis required for division¹⁶, (4) initiation of termination protein synthesis²⁰, (5) onset of sensitivity to penicillin (activation of autolysins)²¹ and (6) attainment of a particular length. (The first visible signs of septation can be detected within a few minutes of this time²².) One or more of these events might therefore be necessary for the increase in rate of elongation to take place.

It has already been suggested^{6,7} that termination of each round of chromosome replication might be a signal for a doubling in the rate of cell elongation. This suggestion stemmed from observations on the relationship between cell volume, length and radius as functions of the transit time, C , taken by replication forks to travel along the chromosome. According to this model the rate of cell elongation would be determined solely by (1) the number of chromosome termini per cell, and (2) the doubling time for cell mass.

To test this hypothesis, we investigated whether an increase in the rate of cell elongation would take place in the absence of chromosome termination. Step-up experiments, such as that

described, provide a sensitive technique for such a determination. We have therefore shifted cells from a poor to a rich medium in conditions in which chromosome termination was prevented. We found that this does not prevent the increase in rate of cell elongation at the same time as in controls.

For this experiment we used a low thymine-requiring strain, B/r/1 T⁻ (ref. 9) which has the advantage that it is able to increase in mass at the control rate for nearly 1 h after removal of thymine, although DNA synthesis stops immediately. (Thymine auxotrophs of the other strains mentioned here could not maintain this rate for so long. Nevertheless, we have observed that their lengths increase in a similar way to B/r/1 T⁻ during this time.) It should be noted that this strain is about 50% longer than the others described here.

Small cells were selected as before from an exponential population in minimal medium plus thymine (generation time 56 min) and transferred to a rich medium plus or minus thymine. After transfer both aliquots grew initially with a mass doubling time of 33 min (Fig. 4), although growth in the thymine-starved aliquot slowed down by about 50 min after transfer. Synchronous division of the control cells took place between 35 and 45 min while the thymine-starved cells showed only a small increase in number. The initial rate of length increase in both cultures was initially that appropriate to the pre-shift growth rate but changed abruptly to the expected higher rate (roughly 3.8 times greater) at about 30 min in both cultures. We consider that this experiment shows that termination of chromosome replication is not required as signal for the observed abrupt increases in rate of cell elongation.

This conclusion is further corroborated by the observation that an increase in the number of chromosome termini per cell,

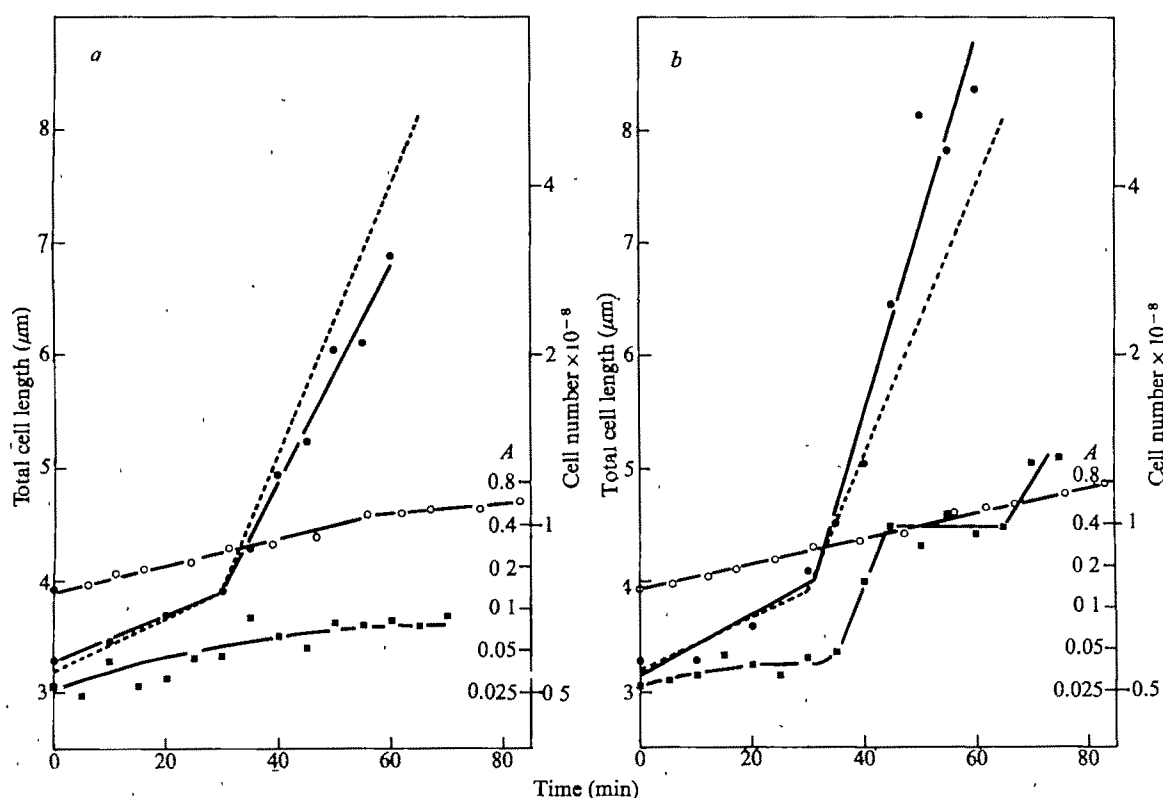


Fig. 4 Increase in total cell length, mass and number (symbols as in Fig. 3) following transfer of selected small cells to a richer medium, *a*, with or *b*, without further chromosome replication. A homogeneous fraction of small cells was selected by sucrose gradient centrifugation of a population of cells of *E. coli* B/r/1 T⁻, growing with a generation time of 56 min in medium 2 + thymine (50 μg ml⁻¹) and reinoculated into medium 7 (Table 1) *b*, with or *a*, without added thymine. In both cases the mass doubling time changed initially to 33 min but growth gradually slowed after about 50 min in the absence of thymine. DNA synthesis in this strain stops immediately after removal of thymine from the medium²³. In the presence of thymine, but not in its absence, synchronous cell division took place between 35 and 45 min and again after 65 min. (A small increase in cell number took place initially in both cultures, due to the separation of a proportion of cell pairs present in the selected fraction.) In both cultures total cell length increased initially at about the same rate and then increased abruptly at about 30 min. (This rate change was slightly higher than predicted in *b* and slightly lower than predicted in *a*, probably due to the general slowing down in growth after prolonged thymine deprivation, but the change in rate is clearly seen in both cases.)

caused by premature termination in the absence of cell growth, also does not affect the rate of cell elongation when growth is resumed. This experiment is described in Table 2.

Model of cell growth and division

We may summarise our observations as follows: (1) Cells divide about 20 min after reaching a particular length, 2Λ , (which is about 2.8 μm for a K12 strain and for one B/r strain), independent of the growth rate of the cells (2) The rate of cell elongation changes at about this same length. If growth conditions remain constant, the rate doubles at this point, whereas if growth conditions have been changed during the course of the preceding cycle, the growth rate changes at this same point to a value proportional to the cell's new rate of mass increase. (3) The critical length, 2Λ , is twice the minimum theoretical length for cells. (4) Termination of chromosome replication, although necessary for subsequent cell division^{17,18}, does not control the change in rate of cell elongation.

A model consistent with these findings is that there is a minimum unit cell, of length Λ μm , which has a fixed number of sites of elongation (perhaps one). Growth in length of such a unit cell takes place at these sites, at a rate proportional to the number of sites, as well as to the growth rate in cell mass, until a new unit length has been completed (at a total length of 2Λ μm). At this point two things happen: septation is initiated between the two completed unit cells, and each of the two units begins to grow independently from new sets of growth sites.

The septation process, once initiated on the completion of the new unit, takes about 20 min to complete, independent of the growth rate. As a result, even though cells always initiate septation at a fixed length, the final length at the time of separation (and hence the average length of the population of cells) will be proportional to the growth rate. Thus, one of the oldest and simplest ideas about the control of cell division, namely that cells always begin to divide whenever they reach a certain critical size is, with minor modifications, seen to be consistent with our observations.

The rate at which each new unit cell grows would seem to be set at the time of its initiation. This rate is proportional to the growth rate in mass of the cell at the time it reaches length

Table 2 Rate of elongation of "preterminated" cells and control cells

Time (min)	Calculated ratio (termination theory)	Observed ratios	
		\bar{L}_p/\bar{L}_{c1}	\bar{L}_p/\bar{L}_{c2}
0	1.00	1.01	1.01
40	1.29	0.97	0.95
60	1.38	—	0.97
80	1.30	0.90	0.96

Ratios of mean cell lengths in synchronous cultures of *E. coli* K12 "M6K", a *leu⁻thyA⁻(dra⁻ or drn⁻)* derivative of MB93 A81 (ref. 23) growing at 30 °C in medium 3 (Table 1). Small cells were selected by sucrose gradient centrifugation²⁴ and either (1) reinoculated into the same medium (control 1) or (2) starved for leucine for 120 min to allow termination of chromosome replication^{16,20} before readdition of leucine and resumption of growth (preterminated cells) or (3) starved for both leucine and thymine for 120 min to prevent termination before readdition of both supplements and resumption of growth (control 2). Synchronous division took place at about 80 min after reinoculation into fully supplemented medium in all three samples. Mean cell lengths were measured at the indicated times after resumption of cell growth (\bar{L}_p for preterminated cells, \bar{L}_{c1} for control 1; \bar{L}_{c2} for control 2). The ratios of these lengths did not change significantly during the course of the experiments. The "calculated ratios" were based on the assumption that the rate of cell elongation would double at termination of chromosome replication. Termination in the control cells was assumed to take place 20 min before division. The maximum ratio of mean cell lengths would therefore be expected at 60 min. DNA synthesis was found to behave as expected, doubling once per cell cycle at the same time in both controls (following readdition of supplements), whereas it continued at the control rate for about 40 min in the leucine-starved cells and then stopped quickly. DNA synthesis in this sample, as expected, did not recommence immediately on readdition of leucine (although growth resumed immediately) but recommenced abruptly after about 20 more min.

2Λ (which is the time when the new sets of growth sites are supposed to be activated). Because the rate of mass increase responds immediately to changes in growth conditions, no matter at which stage of the cycle the change is made¹⁶, the volume of cells changes with growth rate in a way which is different from that which we describe here for cell length. Consequently, cell diameter increases with growth rate such as to accommodate the larger increase in volume than length^{6,7}. The different ways in which growth in mass and in length respond to changes in growth conditions provide an explanation for the observed proportions of cells at different growth rates and also results in the change from one set of proportions to the next being made within a single cell cycle of a change in conditions (see Figs 3 and 4).

Sargent⁸ has recently reported that for several theoretical models of cell growth the length of cells of *Bacillus subtilis* changes in a way which is consistent with a doubling in rate of elongation at a constant cell length that is independent of growth rate. From his data, together with some measurements of our own, we find that this length is also close to twice that calculated for "minimum cells" (zero growth rate) and, interestingly, that this value is very close to that estimated for *E. coli*. The large size, relative to *E. coli*, of *B. subtilis* cells in most growth media can therefore be ascribed entirely to the long duration of the septation process (estimated at about 1 h) in this organism. Sargent also reported that cells of *B. subtilis* change only in length, without change in diameter, at different growth rates⁸. This implies that, unlike *E. coli*, the rate of elongation per growth site is likely to change immediately after a shift in growth rate.

The model of cell growth presented here seems to be inconsistent with the observations on the variation in cell length and width as a function of thymine concentration in cultures of thymine-requiring auxotrophs of *E. coli*^{6,7}. As the thymine concentration is reduced, the replication time of the chromosome is increased, so that termination and cell division are delayed and thus average cell mass is increased. Our present model predicts that this increase in cell volume should be the result solely of an increase in cell length, but the reported measurements show that it is in fact largely due to an increase in cell width. This observation led to the suggestion that the rate of cell elongation is proportional to the number of chromosome termini per cell^{6,7}. Our results, however, show no such relationship. We do not know the reason for this apparent discrepancy.

In our model, septation and the activation of new growth sites take place at a fixed cell length, 2Λ . It is not implied however, that the trigger for these events is cell length *per se*. It may well be some correlated event, such as the completion of the required period of division protein synthesis¹⁶, which we have already suggested as the trigger for the initiation of the final septation process²⁰. But it is not possible to distinguish experimentally between the completion of a required period of protein synthesis and the attainment of the critical length as being the triggering event, because both are achieved at the same time and presumably involve similar biosynthetic reactions. The only correlated event which we have been able to eliminate is chromosome termination, because inhibition of DNA synthesis can be carried out specifically without immediately affecting cell growth. For the moment therefore, we can only point out the correlation and hope that further experiments will help to discover the proximate signal for the initiation of division.

We thank Millicent Masters and John F. Collins for advice.

Addendum: After this manuscript was submitted it came to our notice that N. B. Grover *et al.* have also measured cell length as a function of growth rate in *E. coli* with results which are indistinguishable from ours. They also conclude that their data agree with a model in which the rate of cell elongation doubles at about the time of chromosome termination.

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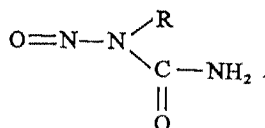
All oxygens in nucleic acids react with carcinogenic ethylating agents

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Over 80% of ethylnitrosourea and ethylnitrosoguanidine modification of nucleic acids is on oxygens. The reactivity of oxygens (other than ribose and phosphate) in single-stranded RNA is: O^2 of C > O^2 of U > O^6 of G > O^4 of U. In double-stranded DNA the order is: O^2 of T = O^6 of G > O^4 of T > O^2 of C. Oxygen reactivity of single-stranded DNA resembles RNA. The glycosidic bond of O^2 -alkylpyrimidines is labilised.

ALKYLATING agents of the general structure



are potent carcinogens which do not require metabolic activation. The most studied of this type of nitroso compound are *N*-methyl-*N*-nitrosourea (MeNU) and *N*-ethyl-*N*-nitrosourea (EtNU). Another group of carcinogens which are also highly mutagenic are also nitroso derivatives, the best known of which are *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine (ENNG).

In recent years it has been found that the *N*-nitroso alkylating agents have an affinity for alkylating the oxygens of nucleic acids, in contrast to the "typical" alkylating agent (for example, dimethylsulphate) which is of low carcinogenicity or mutagenicity and which primarily alkylates ring nitrogens (reviewed by Singer, ref. 1). Following Loveless's suggestion that the O^6 of guanine could be alkylated in nucleic acids², a number of investigators did find that a significant proportion of the total alkylation was O^6 -alkyl G when nucleic acids were treated *in vitro* or *in vivo* with MeNU, MNNG and EtNU³⁻¹¹.

Due to the known lability of the alkyl group of O^6 -alkylguanosine, this derivative, and generally no other, was determined in enzyme digests of RNA rather than after hydrolysis with strong acid or base as customary for *N*-substituted derivatives. In DNA, either enzyme digestion or mild acid depurination was the method generally used to isolate that nucleoside or base without concomitant dealkylation. Singer and co-workers then found that enzyme digests of nucleic acids treated with MeNU or EtNU contained alkyl esters which were derived from alkylation of the internucleotide phosphodiester, and that alkyl phosphotriesters represented about two-thirds of the total bound alkyl groups in DNA and

RNA^{10,11}. Further examination of the alkyl products after enzyme digestion at neutral pH revealed that 10-15% of the alkylation of RNA by EtNU was on the ribose¹².

I now report that the newly described derivative, O^2 -ethylcytidine (O^2 -EtCyd)¹³, is a major product of neutral aqueous reaction of the single-stranded nucleic acids, TMV RNA and M13 DNA, with EtNU and ENNG. In double-stranded salmon sperm DNA this reaction is greatly suppressed and O^2 -ethylation of cytosine is only barely detectable. A further new finding is that both oxygens on uracil and thymine react with EtNU and ENNG. O^2 substitution of U in RNA and T in double-stranded DNA is greater than O^4 substitution, but in single-stranded DNA the O^2 and O^4 of T are equally reactive.

Techniques and their pitfalls

There are at least three reasons why *O*-alkylation of pyrimidines in nucleic acids has been overlooked until now. The first, and probably most important, is that one finds only what one looks for. Almost all of the *O*-alkyl pyrimidines have been prepared for the first time in this laboratory in the last year, and without authentic markers it is difficult to find and identify new products. Second, all *O*-alkyl pyrimidines are labile in acid and alkali so that losses (up to 100%) occur during the degradation of a nucleic acid unless neutral enzymatic digestion is used. Furthermore, separation of products in a digest must also be done under conditions which preserve the *O*-alkyl linkage. Third, mere coincidence of radioactivity with an ultraviolet absorbing marker in any system does not mean that the radioactive substance is completely or even partly identical with the marker.

The techniques used in this work which enabled us to avoid these pitfalls and to separate and quantitate the new derivatives discussed in this paper are all given in detail in preceding papers. Briefly they are: (1) the use of snake venom phosphodiesterase and phosphatases at pH 7.2 to digest alkylated polynucleotides and nucleic acids to nucleosides^{10-12,14,16}; (2) one-dimensional paper chromatography using butanol-ethanol-H₂O (80:10:25) (Solvent 1) to separate groups of possible products¹²⁻¹⁸; (3) additional separation techniques, for each group of possible products eluted from chromatograms, utilising the unique characteristics of the individual alkyl derivatives. For example, electrophoresis to separate products with different pK_a s^{15,16} or chromatography in borate-containing solvents to separate *O*-alkylnucleosides^{13,14}; (4) preparation and use as markers of all *N*- and *O*-monoethylated nucleosides¹⁰⁻¹⁸, and determination of their stability in conditions used for enzyme digestion, the R_f values in several neutral chromatographic systems, electrophoretic behaviour, rate of

acidic dealkylation, and so on; and (5) awareness of the fact that alkylation of pyrimidines in nucleic acids can diminish the stability of the glycosidic linkage.

Proof of formation of O^2 -ethylcytidine and O^2 -ethyldeoxycytidine

When alkylated single-stranded RNA, and poly(C) were enzyme-digested to nucleosides and chromatographed in a

neutral solvent, peaks of radioactivity co-chromatographed with authentic O^2 -ethylcytidine (Fig. 1) (data not shown for poly(C)). In the case of TMV RNA treated with EtNU or ENNG about 10% of the total radioactivity was in this area (A) (as shown in Fig. 1c and d), while with diethylsulphate (Et_2SO_4) reaction, no peak was exactly coincident with the marker (Fig. 1f) but about 2% of the radioactivity could be assigned to the marker area.

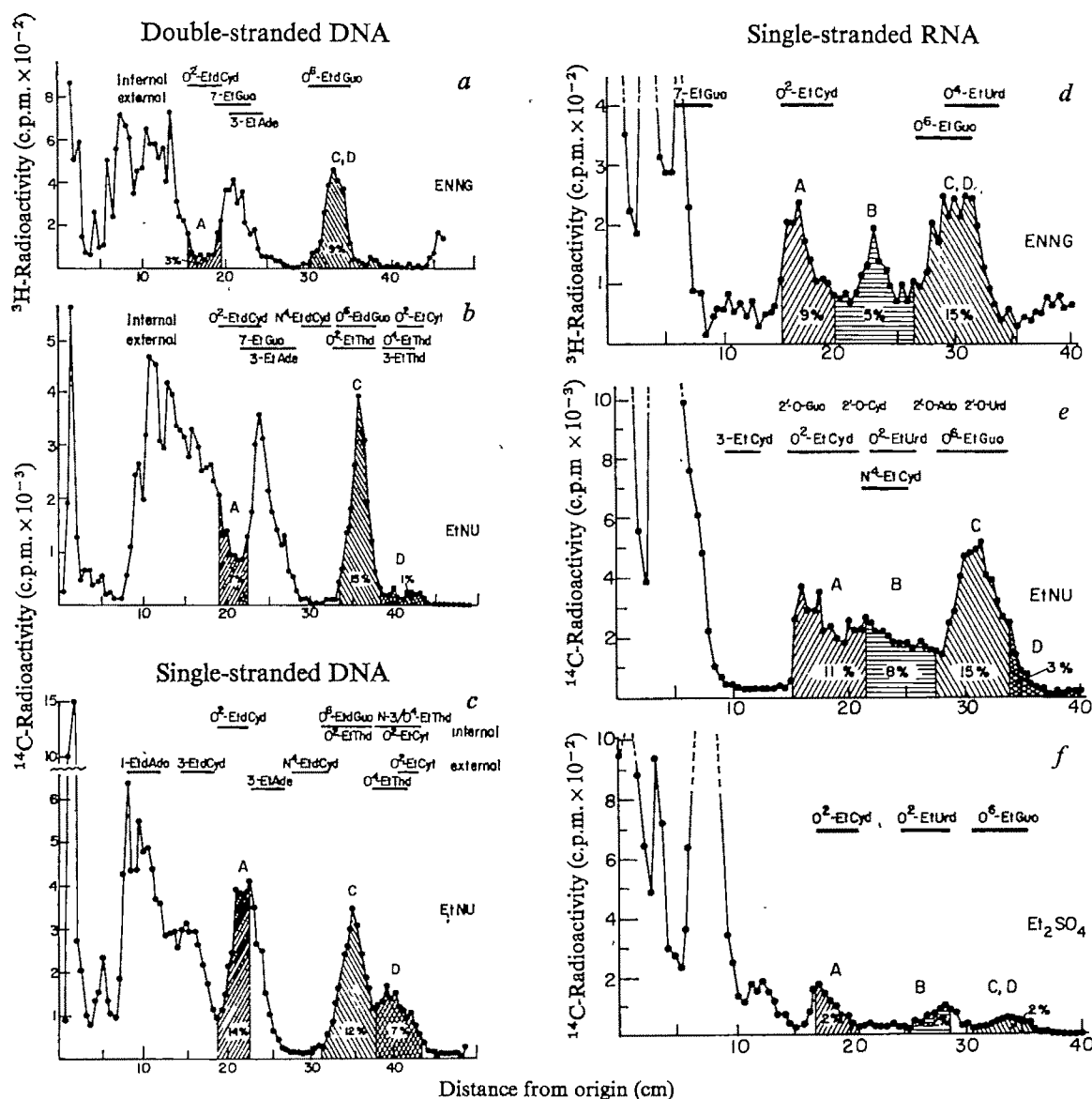


Fig. 1 Radioactivity profiles of chromatograms of enzyme digests of ethylated nucleic acids. 0.5–1 mg TMV RNA, salmon sperm DNA and M13 DNA were reacted in pH 6.1 or pH 7.3 cacodylate buffer 37 °C, 1–3 h, with ^{14}C -ethylnitrosourea (Farbwerke Hoechst AG, 4.1 Ci mol⁻¹) or ^3H -ethylnitrosoguanidine (Amersham/Searle, 110 Ci mol⁻¹) or ^{14}C -diethylsulphate (ICN, 4.09 Ci mol⁻¹). The methods are described in detail in earlier papers^{10,11}. After precipitating with ethanol until constant specific activity was obtained, the specific activity of ethylated nucleic acids varied from 20–400 c.p.m. μg^{-1} . The lowest extent of reaction was consistently obtained with ethylnitrosoguanidine. The percent alkylation with each reagent was very similar for RNA and DNA^{10,11}. Ethylated RNA (50–200 μg) was digested with snake venom phosphodiesterase, acid and bacterial alkaline phosphatases using method I described by Kusmierek and Singer¹⁴. 100–750 μg ethylated DNA in 0.1 M pH 7.2 Tris buffer containing 0.01 M MgCl_2 was digested with an equal weight of deoxyribonuclease, 37 °C, 4 h, then further digested with equal weights of snake venom phosphodiesterase, acid and bacterial alkaline phosphatases, 37 °C, 18 h. Singer and Kusmierek¹⁴ have reported that these neutral digestion conditions permit recovery of labile O -alkyl nucleosides and other alkylated nucleosides or bases such as 7-alkyl G and 1-alkyl A which are ring-opened and rearranged, respectively, at higher pHs. After adding ultraviolet-absorbing markers (prepared in this laboratory^{10–18,20}), digests of alkylated RNA or DNA were applied to Whatman 3MM and developed 16–20 h in butanol–ethanol–water (80:10:25). The positions of the marker nucleosides or bases were noted, and the entire paper was cut into 0.5-cm strips (to which were added 5 ml toluene containing 14.3 g Omnifluor/3 kg toluene) for radioactivity counting. Panels a–f are typical profiles and in each panel the type of nucleic acid and reagent are given. The bars above the chromatograms represent the ultraviolet-absorbing areas of each marker and in general the internal and external markers have the same R_f . In e the approximate positions of 2'- O -ethylnucleosides are also shown, based on their known R_f values¹⁴. O^2 -EtdCyd or O^2 -EtdCyt are termed 'A'; the area coinciding with O^2 -EtdUrd, 'B'; the area coinciding with O^2 -EtdGuo, 'C'; and the area coinciding with O^2 -EtdThd and O^2 -EtdCyt or O^4 -EtdUrd, 'D'. The percent of total radioactivity in each area is shown in the figures. After counting, paper strips from these and other areas were washed several times in toluene, dried and eluted with either water or methanol, then treated in various ways in order to separate the possible derivatives in each area (see text and Figs 2 and 4–6).

O^2 -EtCyd does not separate clearly in Solvent 1 from $2'$ - O -EtGuo or 1 -EtGuo, and peak A could contain these two derivatives. However, both Guo derivatives have a pK_a of ~ 2 (ref. 19) while O^2 -EtCyd has a $pK_a > 9.5$ ¹³. Consequently electrophoresis at $pH \sim 9$ was found to separate O^2 -EtCyd not only from derivatives of "low pK_a " but also from all other ethyl derivatives including those with a "high pK_a " such as 1 -EtAdo and 3 -EtCyd ($pK_a \sim 8.5$)¹⁹. Figure 2a illustrates that most of the radioactivity (from peaks dA and eA) coincides with O^2 -EtCyd upon electrophoresis in 0.1 M ammonium carbonate. The small peak of low pK_a was shown to be $2'$ - O -EtGuo ($\sim 1.8\%$ of total alkylation) when re-chromatographed in a borate-containing solvent¹⁴. Further proof that peak A was primarily O^2 -EtCyd was that the radioactivity from the high pK_a area of the electrophoresis (1) co-chromatographed with the marker and could be separated from 1 -EtGuo, the derivative with the closest R_f , by long chromatography in solvent 1 (Fig. 2b), and (2) the ^{14}C -labelled ethyl group was hydrolysed in N HCl at the same rate as the marker was dealkylated to cytidine.

The ethylation of the O^2 of dCyd in DNA was studied using double-stranded salmon sperm DNA and single-stranded M13 DNA. As seen in Fig. 1a and b, enzyme digests of ENNG and EtNU-treated salmon sperm DNA did not exhibit a peak of radioactivity co-chromatographing with O^2 -EtdCyd. The radioactivity, termed peak A, which really represented a minimum, was analysed using similar methods to those used to detect O^2 -EtCyd and it was found that O^2 -EtdCyd was less than 0.3% of the total radioactivity. The large peak moving slightly ahead of the marker O^2 -EtdCyd was identified by two-dimensional chromatography¹¹ as containing 7 -EtGua and 3 -EtAde (3:1); both bases were also in peak A and their presence illustrates the liability of the glycosidic bond of alkylpurine deoxynucleosides even at $pH 7.2$.

In studying the stability of the ethyl group in O^2 -EtdCyd (prepared from dCyd as described for the preparation of O^2 -EtCyd from Cyt¹³) it was found that heating in 0.1 N HCl at $70^\circ C$ for 30 min or at $pH 7.0$, $100^\circ C$ 1 h, caused complete depyrimidation without loss of the ethyl group and the product was O^2 -ethylcytosine (Fig. 3). These conditions are often

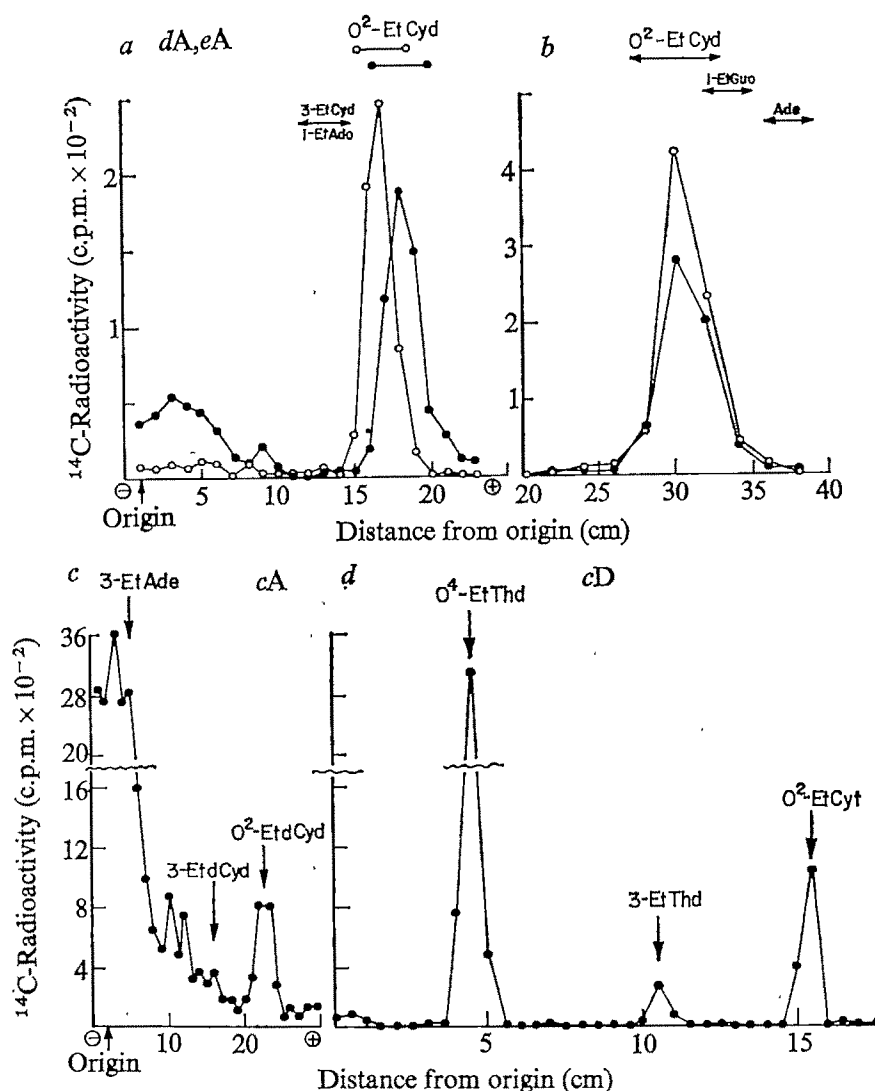


Fig. 2 Separation and identification of O^2 -EtCyd or O^2 -EtdCyd and O^2 -EtCyt. *a*, Superimposed radioactivity profiles of electrophoretograms of peak A from Fig. 1d and e (termed dA, eA). Material eluted from the O^2 -EtCyd area of EtNU- and ENNG-treated TMV RNA was separately electrophoresed in 0.1 M $(NH_4)_2CO_3$ ($pH \sim 9$)¹³. The electrophoretogram was cut in 101 cm strips and the radioactivity counted. The open circles are from dA and the closed circles from eA. The position of the internal O^2 -EtCyd for each electrophoretogram is similarly indicated. The mobility, at this pH , of O^2 -EtCyd was higher than any other ethylated nucleoside as shown by the separation of O^2 -EtCyd from 3 -EtCyd (pK_a 8.4) and 1 -EtAdo ($pK_a \sim 8.5$)¹⁹. *b*, Superimposed radioactivity profiles of a portion of chromatograms of the material co-electrophoresing with O^2 -EtCyd in *a*. After elution, samples were chromatographed in the same solvent as in Fig. 1 except that the chromatogram was developed 48 h. O^2 -EtCyd was internal while 1 -EtGuo and Ade were used as external markers since with 16–20 h chromatography these derivatives do not separate from O^2 -EtCyd (data not shown) while with longer development their movement differs. *c*, Radioactivity profile of an electrophoretogram of peak A from Fig. 1c (termed cA). Material eluted from the O^2 -EtdCyd area of EtNU-treated M13 DNA was electrophoresed in 0.1 M $(NH_4)_2CO_3$ ($pH \sim 9$). O^2 -EtdCyd is completely separated from 3 -EtAde (pK_a 6.5) and 3 -EtdCyd (pK_a 8.5) as well as all derivatives with a $pK_a < 6.5$. The radioactivity co-electrophoresing with O^2 -EtdCyd had the same acid stability as the marker. *d*, Radioactivity profile of a silica gel thin-layer chromatogram of peak D from Fig. 1c (termed cD). This peak from EtNU-treated M13 DNA which contained ultraviolet-absorbing markers of O^2 -EtThd, 3 -EtThd and O^2 -EtCyt was developed in acetone-benzene (2:1), then cut in 0.5 cm strips and the radioactivity counted.

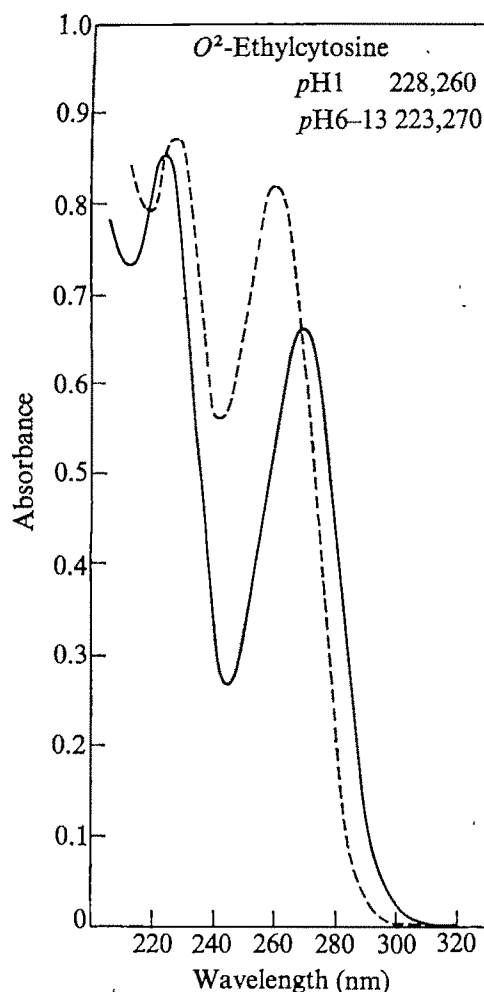


Fig. 3 Ultraviolet absorption spectra of O^2 -ethylcytosine at pH 1 (---) and pH 6-13 (—). The absorption maxima are given in the figure. O^2 -Ethylcytosine was prepared by the following method. To 30 mg cytosine in 10 ml methanol was added a solution of diazoethane in ether prepared according to the procedure of Robins *et al.*³² for the preparation of diazomethane (solution A). Several additions of several millilitres each were needed to saturate the solution. The ether was evaporated off and the remaining solution chromatographed on Whatnam 3MM in butanol-ethanol-water (80:10:25) to separate the several derivatives. O^2 -Ethylcytosine has in this system an R_f 0.88 and moves ahead of all other ultraviolet absorbing compounds. The yield from 30 mg cytosine is about 300 absorbancy units. O^2 -methylcytosine was prepared with a lesser yield using diazomethane. The methyl derivative, obtained by a different method, has been described by Sukhorukov *et al.*³⁴, although the second λ_{max} (228 in acid and 223 in alkali) was not shown by them probably due to impurities in their sample. The reported pK_a of O^2 -methylcytosine is 5.41 (ref. 36).

used for depurinating alkylated DNA and would be likely to depyrimidate O^2 -EtdCyd. Since a substantial proportion of 7-EtdGuo and 3-EtdAdo was depurinated during pH 7.2 enzyme digestion, it seemed reasonable to suspect that, in addition to 7-EtGua and 3-EtAde, O^2 -EtCyt would also be present and by R_f would be in peak D. This was shown by electrophoresis of peak D (Fig. 1c) at pH 5.5, where O^2 -alkylcytosine which has a reported pK_a of 5.4 (ref. 19) migrates while the other possible products, O^4 -EtThd and 3-EtThd, do not move at this pH. Although O^2 -EtCyt could be identified, the amount was extremely low.

Identification of O^2 -EtdCyd and O^2 -EtCyt was unequivocal in EtNU-treated single-stranded M13 DNA. First, although peak A (Fig. 1c) was not completely coincident with O^2 -EtdCyd, the radioactivity sharply increased in the area of the marker,

rather than decreasing as was the case for double-stranded DNA. Electrophoresis at pH ~9 of the shaded area in peak A clearly resolved O^2 -EtdCyd from other possible neighbouring ethyl nucleosides of pK_a 6.5 and pK_a ~8.5 (Fig. 2c), and there was a distinct peak of radioactivity co-electrophoresing with the authentic nucleoside. On the basis of earlier experiments it was expected that O^2 -EtCyt would also be formed as a result of depyrimidation during enzyme digestion. Peak D contained as internal markers O^2 -EtThd, 3-EtThd and O^2 -EtCyd, which were resolved by silica gel thin layer chromatography. A distinct and sharp peak of radioactivity coincided with O^2 -EtCyt (Fig. 2d) and other similarly sharp peaks were found with the other two markers.

Proof of formation of O^2 - and O^4 -ethyluridine and O^2 - and O^4 -ethylthymidine

The R_f of O^2 -EtUrd in the chromatographic system used in Fig. 1 is very similar to the R_f s of 2'- O -EtCyd and N^4 -EtCyd and the peak from RNA digests designed as 'B' in Fig. 1d-f could contain all three derivatives. Similarly, O^4 -EtUrd is coincident with 3-EtUrd (peak D) and not completely separable from O^6 -EtGuo and 2'- O -EtUrd (peak C) (Fig. 1d-f). Several techniques were used to resolve the derivatives in peaks B, C and D; the most useful of these were electrophoresis in pH 3.0 sodium citrate to separate O^2 -EtUrd (pK_a < 1) from N^4 -EtCyd and 2'- O -EtCyd (pK_a ~4.2)¹⁹ and thin-layer chromatography²⁰ which can separate O^4 -EtUrd from O^6 -EtGuo, 3-EtUrd and 2'- O -EtUrd in peaks C and D. Figures 4b and 5b illustrate these separations. The areas of radioactivity co-chromatographing with O^4 -EtUrd (shaded area of Fig. 4b) and co-electrophoresing with O^2 -EtUrd (shaded area of Fig. 4a) were each eluted and in both cases all radioactivity was volatilised after treatment with N HCl (100 °C, 60 min) and the ultraviolet-absorbing markers were converted to uridine, thus confirming that the original derivatives were oxygen substituted.

O^2 -EtThd has the same R_f as O^6 -EtdGuo (peak C) (Fig. 1a-c) and the two nucleosides also behave similarly in that both lose their ethyl group upon hydrolysis in N HCl at 100 °C, 60 min. However, thin-layer chromatography²⁰ clearly separates them (Figs 5a and 6a) as well as O^4 -EtThd and 3-EtThd which are mainly in peak D (Fig. 6b). Thin-layer chromatography of

Table 1 Proportion of ethyl products from hydrolysates of nucleic acids reacted with ^{14}C -ethylnitrosourea

Derivative	TMV RNA	M13 DNA	Salmon sperm DNA
Total ethylation* (%)			
O^6 -EtGuo or O^6 -EtGuo	5†	7	7‡
O^2 -EtUrd	7		
O^2 -EtThd		5	6
O^4 -EtUrd	3		
O^4 -EtThd		5	1.4
O^2 -EtCyd	8		
O^2 -EtdCyd + O^2 -EtCyt		3	0.5
Total	23%	20%	15%
2'- O -EtAdo, -Guo, -Cyd, -Urd	12±2§	—	—
Ethylphosphotriester	65¶		70
N-Ethyl	16		17

*See Fig. 1 for methods. The per cent ethylation in different experiments varied from 0.5 to 1.5%.

†Singer and Fraenkel-Conrat¹⁰ reported 12% O^6 -EtGuo on the basis of coincidence of radioactivity with the authentic marker and volatility of most of the ethyl groups in N HCl 100% 1 h. The present data show that this peak also contains O^4 -EtUrd and 2'- O -EtUrd.

‡Sun and Singer¹² reported 12% O^6 -EtGuo on the same basis as footnote †. The present data, if O^2 -EtThd and O^6 -EtdGuo are added, give a similar value for this peak.

§Data from Singer and Kušmirek¹³.

¶Data from Singer and Fraenkel-Conrat¹⁰. The amount of esterification was determined by distillation of ethanol after N HCl hydrolysis. Ethanol calculated to be derived from O^6 -EtGuo was subtracted. If now the volatile ethyl groups on the uridine ring are subtracted as well, the calculated amount of riboalkylphosphotriester is about 54%.

||Data from Sun and Singer¹².

combined peaks C and D further illustrates these separations (Fig. 5a). One additional proof of the identity of O^3 -EtThd is the great instability of both the alkyl and the glycosidic bond²⁰; thus hydrolysis in 0.1 N HCl at 70 °C for 30 min almost quantitatively converts this derivative to thymine, while O^6 -EtdGuo is only depurinated, becoming O^6 -EtGua.

O^4 -EtThd and 3-EtThd (peak D) do not separate from O^2 -EtCyd in solvent 1 although the R_f s are somewhat different (Fig. 1b and c). They all separate completely upon thin-layer chromatography, with O^2 -EtCyt moving ahead of the other derivatives (Fig. 2d). In addition, O^2 -EtCyt ($pK_a \sim 5.4$) can

basis of its symmetry and sharpness, contains two major and one minor component (Fig. 6a).

There are two points to be noted before quantitative conclusions are drawn from the data on the figures and Table 1. First, although the alkylated nucleic acids were digested for long periods of time with large amounts of enzymes, from 10 to 40% of the total radioactivity remained at the origin. This material, which was not due to generally incomplete digestion since there was no associated ultraviolet absorbancy, did not elute from the paper even with 0.1 N HCl or long incubation at 37 °C. Since the origin material is labelled, it obviously

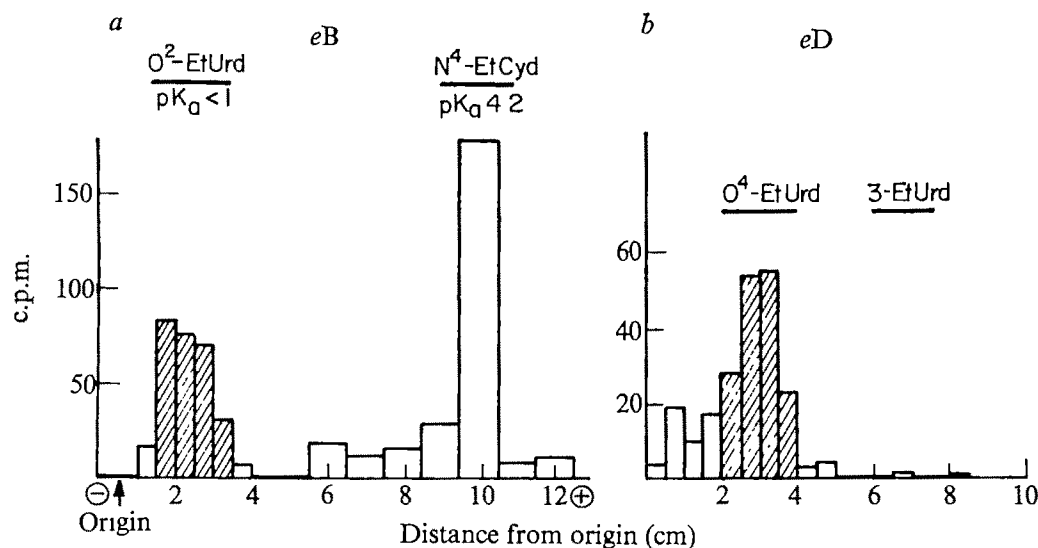


Fig. 4 Separation and identification of O^2 -EtUrd and O^4 -EtUrd. *a*, Radioactivity profile of an electropherogram of peak B from Fig. 1e (termed eB). Material eluted from the O^3 -EtUrd area of EtNU-treated TMV RNA was electrophoresed in 0.1 M pH 3 sodium citrate buffer. O^3 -EtUrd does not migrate at this pH while nucleosides which also could be in peak B, for example, N^4 -EtCyd, move very far. *b*, Radioactivity profile of a silica gel thin-layer chromatogram of peak D from Fig. 1e (termed eD). Material eluted from the O^4 -EtUrd area of EtNU-treated TMV RNA, which also contained a marker of 3-EtUrd, was chromatographed using benzene-acetone (2:1). See Fig. 5b for separation of O^4 -EtUrd from O^6 -EtGuo, $2'$ - O -EtUrd, and 3-EtUrd.

be separated from the thymidine derivatives by electrophoresis in pH 5.7 formate buffer.

Quantitative aspects of *O*-ethylation

In Fig. 1, the percentages given in shaded areas A–D refer to percentage of total recovered radioactivity. In the case of RNA treated with ENNG or EtNU, most of the radioactivity in these areas could be assigned to a known *O*-alkyl derivative. Thus peak A is essentially O^3 -EtCyd (Fig. 2), peak B is about half O^2 -EtUrd (Fig. 4a), peak C contains O^6 -EtGuo and $2'$ - O -EtUrd while peak D is O^4 -EtUrd (Fig. 4b and Fig. 5b). Diethylsulphate-treated RNA (Fig. 1f) was very low in all these derivatives and none of these peaks contained more than 0.5% (of the total radioactivity) of an identifiable derivative. Nevertheless, O^3 -EtCyd was identified in peak A and O^6 -EtGuo and O^4 -EtUrd in peaks C, D.

EtNU-treated single-stranded DNA resembled EtNU-treated single-stranded RNA in the chromatographic profile and about 10% of peak A (Fig. 1c) is O^2 -EtdCyd (Fig. 2c). Peak C is about 40% O^2 -EtThd, 55% O^6 -EtdGuo and the remaining 5% is O^4 -EtThd coming from peak D where it is a major component. Peak D comprises 70% O^4 -EtThd, 24% O^2 -EtCyt and 6% 3-EtThd.

Area A of EtNU- and ENNG-treated salmon sperm DNA (Fig. 1a and b) contains primarily N-ethyl products with a trace of O^2 -EtdCyd. Peak C is almost equally divided into O^3 -EtThd and O^6 -EtGuo (Fig. 6a) while peak D is mostly O^4 -EtThd (Fig. 6b). It also can be seen that neither O^4 -EtThd nor O^4 -EtUrd is totally separated from peak C. Another, and more important, fact to be noted, is that a peak, such as C in Fig. 1b, which might be surmised to be homogeneous on the

represents bound alkyl groups and cannot be disregarded. But at this time I have no further information. (One possibly pertinent point is that alkylated poly(U) and poly(C) are almost completely digested using the same enzymes as judged by the fact that less than 5% of the radioactivity is at the origin.) All percentages of known derivatives are based on total radioactivity on the paper and do include the origin. Consequently it is possible that any derivative may remain partly in an enzyme-resistant oligonucleotide at the origin.

Second, *O*-alkyl derivatives are relatively labile and particularly in the case of DNA the percentage of O^2 -EtThd and O^3 -EtdCyd should be taken as minima since these two derivatives are depyrimidated easily and may be lost during reaction.

Given these caveats, Table 1 presents data for EtNU-treated nucleic acids, averaged from several experiments, which show that O^3 -EtUrd or O^3 -EtThd is a major derivative in EtNU-treated TMV RNA, salmon sperm DNA and M13 DNA. O^4 -EtUrd or O^4 -EtThd is formed in lesser amounts but still exceeds the amount of most N-ethyl derivatives. O^3 -EtCyd is formed to a greater extent than O^6 -EtGuo in EtNU- or ENNG-treated TMV RNA. Formation of this derivative is suppressed in double-stranded DNA but when the DNA is single stranded, considerably more reaction takes place. Data for the alkylation products of ENNG-treated nucleic acids are very similar and are not given in tabular form.

A summary of the relative reactivity of various sites in single- and double-stranded nucleic acids treated with EtNU or ENNG is as follows:

Double-stranded DNA:

Phosphate > N^7 -G > O^3 -T, O^6 -G, > N^3 -A > O^4 -T > O^2 -C, other N-

Single-stranded RNA:

Phosphate > Ribose, $N7-G > O^2-C > O^2-U, O^6-G > O^4-U > N1-A, N3-C > \text{other } N$

The two homo-polynucleotides which were treated with the same reagents are also modified primarily on the oxygens and the data are given below for comparison. The work on poly(U) is published separately¹⁴ but the data for poly(C) are from the present series of experiments.

Poly(U): Phosphate > $O^4 > O^2$, Ribose > $N3$

Poly(C): $O^2 > N3, N^4 > \text{Phosphate} > \text{Ribose}$

We have routinely in the recent past used $NHCl$ volatility as a semi-quantitative test for O -alkylation in RNA and $HClO_4$ volatility for O -alkylation in DNA. The alkyl groups of all of the derivatives discussed in this paper are hydrolysed by the HCl treatment except for ribose alkyl nucleosides which require $HClO_4$. Therefore those alkylating agents which do not with acid yield an alcohol may rather safely be presumed not to modify oxygens significantly. As an example, Singer and Fraenkel-Conrat¹⁰ reported 1% ethanol upon HCl hydrolysis of Et_2SO_4 -treated RNA and now Fig. 1f illustrates that little O -alkylation of pyrimidines occurs, thus serving to validate analytical data obtained with alkylsulphate and alkylalkanesulphonates before our knowledge of O -alkylation of pyrimidines^{10,11}.

Possible biological consequences of O -alkylation

The study of the alkylation of nucleic acids started from the observations that alkylating agents such as mustard gas were

inactivating to DNA containing viruses²¹ and that mustard gas was mutagenic²². The principal site of reaction with mustard gas and many other alkylating agents was found to be the $N7$ of guanine¹ and from then on the presence of 7-alkylguanine was presumed to be the significant event in causing a biological effect. Since alkylation of the $N7$ did not change the base-pairing properties of $G^{23,24}$, its effects in DNA were generally attributed to the rapid depurination of 7-alkyl G which further led to chain breakage by β -elimination.

As time went on the simple relationship between the amount of 7-alkylguanine and biological change eroded. This became particularly apparent when alkylation-induced carcinogenesis was studied and ethylating agents were more carcinogenic in rats than the analogous methylating agents while the amount of 7-ethyl G was a small fraction of the amount of 7-methyl G¹.

When the highly carcinogenic N -nitroso compounds were used to modify nucleic acids *in vitro* or *in vivo* another derivative was found, O^6 -alkylguanine, which represented a few per cent of the total alkyl groups³⁻¹¹. When reactions were performed with alkylating agents of low carcinogenicity, this derivative was not detected or was present in trace amounts^{3,4,6,10,11,25}. Thus the effects of the presence of O^6 -alkylguanine became of prime interest since there seemed to be a correlation between its formation and the carcinogenicity of the alkylating agent. In contrast to 7-alkyl G, O^6 -alkyl G in polymers has been shown to mispair²⁴ and thus could be considered mutagenic. There is also good evidence that there is an enzymatic excision of O^6 -alkyl G from DNA²⁶ and in rats given MeNU or EtNU under conditions where only brain tumours develop, O^6 -alkyl G is removed from the brain very much more slowly than from

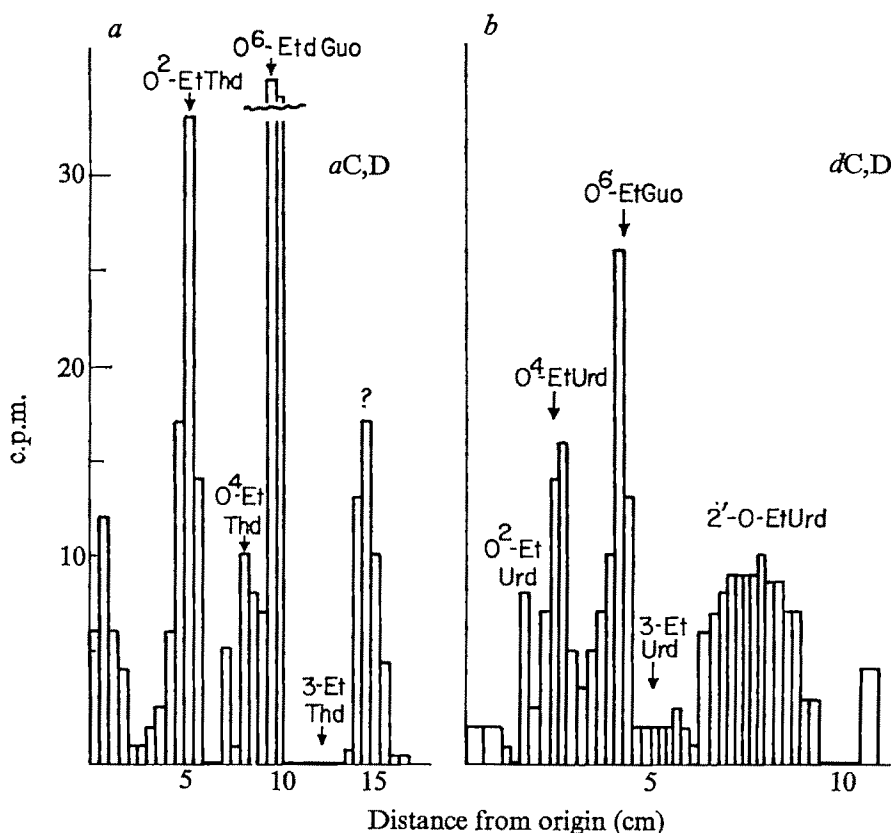


Fig. 5 Separation and identification of O^6 -EtdGuo, O^2 -EtThd and O^4 -EtThd from DNA and the corresponding ribo derivatives from RNA. Radioactivity profile of silica gel thin-layer chromatograms developed with benzene-acetone (2:1). The positions of internal ultra-violet absorbing derivatives are shown by arrows. *a*, Combined peaks C, D from Fig. 1a (termed *aCD*). This material from ENNG-treated salmon sperm DNA contained internal markers of O^2 -EtThd, O^4 -EtThd, O^6 -EtdGuo and 3-EtThd. *b*, Combined peaks C, D from Fig. 1d (termed *dCD*). This material from ENNG-treated TMV RNA contained internal markers of O^4 -EtUrd, O^6 -EtGuo, 3-EtUrd and 2'-O-EtUrd.

other tissues which do not develop neoplasms^{8,9,27-31}. It can be postulated that neoplasms develop in the brain because O^6 -alkyl G remains in the DNA and mispairing occurs.

The same reagents which alkylate the O^6 of G have been shown in this paper to react with all oxygens of the pyrimidines (Table 1). In quantitative terms the O^3 of T in double-stranded DNA reacts to the same extent as does the O^6 of G. Since the alkylation of the O^3 position of T greatly labilises the glycosidic linkage, it is likely that O^3 -alkyl T may depyrimidinate spontaneously or enzymatically leaving a gap which may or may not

or translation. Whether carcinogenesis can be explained solely in terms of mutation is doubtful since mutation is a frequent event and easily produced in cells while carcinogenesis is rare and not yet a test-tube phenomenon.

In conclusion, the *N*-nitroso alkylating agents are powerful carcinogens and alkylate all oxygens in nucleic acids. Other alkylating agents of lesser carcinogenicity do not have an affinity for oxygens. It is tempting to hypothesise that the carcinogenicity of an alkylating agent can be assessed by its reactivity towards oxygens, but I believe this is still premature.

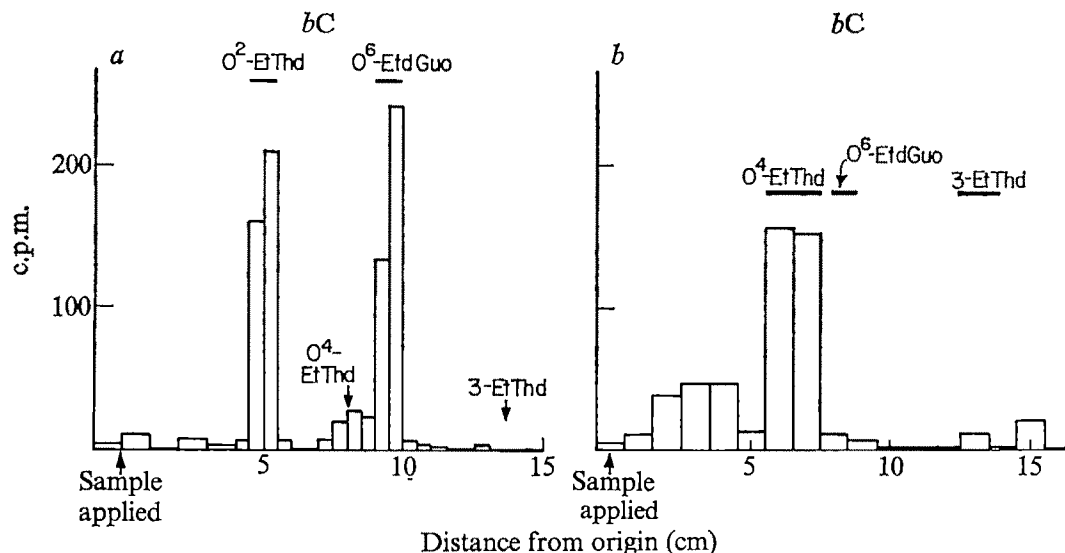


Fig. 6 Separation and identification of O^6 -EtdGuo, O^2 -EtThd and O^4 -EtThd. Radioactivity profiles of silica gel thin-layer chromatograms developed with benzene-acetone (2:1). Peaks C and D, from EtNU-treated salmon sperm DNA (termed 2C and 2D) were separately chromatographed. The position of the internal ultraviolet-absorbancy derivatives are shown by bars or arrows. *a*, Peak *bC* containing internal markers of O^2 -EtThd, O^4 -EtdGuo and 3-EtThd. See Fig. 5, left panel, for a similar chromatogram of combined peaks C and D.

be repaired. In O^2 -alkyl U or T there is no proton at the *N*-3 which means that normal base-pairing with A cannot occur.

Alkylation of the O^4 of U or T may also lead to mispairing since these derivatives also cannot pair normally to A but instead may tend to base pair with G and thus be considered pro-mutagens. While the amount of O^4 -EtThd is relatively low in double-stranded DNA, it is considerable in single-stranded nucleic acids. This is perhaps the place to emphasise that the study of alkylation of "double-stranded" DNA must also consider that replicating DNA contains single-stranded segments and this portion of the molecule will react in a similar manner to any single-stranded polynucleotide, be it RNA or DNA.

The O^3 of C in double-stranded DNA is quite unreactive but in single-stranded DNA the reactivity is greatly increased, and in RNA the amounts of O^3 -alkyl C exceeds that of any derivative modified on the base. This modification in DNA destabilises the glycosidic bond and even with the most gentle handling, half of the derivative is found as the base, O^3 -ethylcytosine. This readiness to depyrimidinate may be occurring continually in the cell with the effect of creating deletions. In RNA where the glycosidic linkage is stable, O^3 -alkylation of C forms a quaternary structure and it is predicted that there will be random mispairing of bases.

Two other significant oxygen alkylations by *N*-nitroso compounds should be mentioned although they are the subjects of separate papers. In RNA ribose is alkylated¹², a modification which may affect mRNA function, and in both DNA and RNA alkyl phosphotriesters are formed (50–70% of all alkylation)^{10,11} which we have shown to be lethal³². Thus all types of alkylation of a nucleic acid are likely to have biological consequences.

These are probably all related in some way to genome mutation or messenger alteration, that is, a change in transcription

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letters to nature

Direct test of the constancy of fundamental nuclear constants

THE possibility that fundamental nuclear constants may vary slowly while the Universe expands has been discussed by several authors¹⁻⁵. I try here to show that the well known resonance properties of the 'heavy nucleus plus slow neutron' system make it a sensitive 'receiver', sharply tuned to the current values of nuclear constants.

What are the restrictions, imposed by experiment that during the time interval ΔT the resonance energy shift had not exceeded ΔE_{exp} ? Simple estimates of residual interaction matrix elements suggest that for the overwhelming majority of compound nucleus resonances one should expect their shifts to be not less than the single-particle resonance shift ΔE_0 . The latter is connected with the relative change in strong coupling constant g_s ,

$$\frac{\Delta E_0}{V_0} \approx \frac{\Delta g_s}{g_s} \quad (1)$$

where V_0 denotes the depth of the nuclear potential well. Assuming $dg_s/dt = \text{constant}$, we get a restriction

$$\frac{1}{g_s} \left| \frac{dg_s}{dt} \right| \lesssim \frac{1}{\Delta T} \frac{\Delta E_{\text{exp}}}{V_0} \quad (2)$$

The positions of many low lying resonances have been known to an accuracy of 10^{-3} eV for quite a time⁶. Assuming $V_0 \approx 50$ Mev (ref. 7) and $\Delta T \approx 10$ yr we derive

$$\frac{1}{g_s} \left| \frac{dg_s}{dt} \right| \lesssim 2 \times 10^{-13} \text{ yr}^{-1} \quad (3)$$

which is the same result as that established by Davies on the basis of Dyson's cosmological argument⁵.

The Coulomb force increases the average internucleon distance by $\sim 2.5\%$ for $A \approx 150$ (ref. 7). Thus we obtain an estimate for the Coulomb coupling constant α 20 times higher than

Table 1 Comparison of upper bounds of the variation of nuclear constants

	Dyson, Davies	Present work
$1/g_s dg_s/dt (\text{yr}^{-1})$	2×10^{-13}	5×10^{-13}
$1/\alpha d\alpha/dt (\text{yr}^{-1})$	2×10^{-14}	10^{-17}
$1/g_w dg_w/dt (\text{yr}^{-1})$	10^{-10}	2×10^{-13}

from equation (2). The weak interaction contribution to the total energy of the nucleus is $\sim 10^{-8}(\mu/m)^2$, where μ and m are the pion and nucleon mass, respectively⁸. The upper bound on the time variation of the weak coupling constant g_w is therefore 5×10^6 times higher than for g_s .

The low lying resonance parameters determine the capture cross section for slow neutrons. So data on thermal cross section values in the remote past are of great interest. The recently discovered traces of ancient (1.8×10^9 yr old) natural nuclear reactors in the uranium deposits of Oklo (Gabon, West Africa)^{9,10} have proved to be important in this respect.

The isotopic composition of Sm and Eu has been measured¹¹ for samples in the reactor core, irradiated by an independently determined¹² integrated flux of thermal neutrons $\Phi t \approx 10^{21}$ neutrons cm^{-2} . Given Φt and fission yields one can determine the capture cross-section values $\approx 1.8 \times 10^9$ yr ago. Three standard deviations give the possible range of the cross-section variation, which is connected with the resonance energy shift through the Breit-Wigner formula. One is thus led to the restriction $|\Delta E_{\text{exp}}| \lesssim 0.05$ eV, and to the estimates of the upper bounds on the variation of fundamental nuclear constants shown in Table 1 along with the earlier limits of Dyson and Davies.

These estimates seem to exclude all variants of nuclear constants change based on Dirac's 'Large Numbers Hypothesis'¹. It is, however, desirable to obtain as strict bounds on ΔE_{exp} as possible. Precise measurements of the isotopic shifts for all rare-earth fission products in the reactor core are desirable in this respect.

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Why measure astrophysical X-ray spectra?

MANY of the interesting results of X-ray astronomy such as the presence of compact sources in close binary systems, have been derived from light curve studies¹, obtained with quite simple detectors. On the other hand, a high resolution spectrometer, one of the most sophisticated pieces of instrumentation, is almost invariably included in solar X-ray satellites, and is used increasingly in cosmic studies². Here we wish to stress that even spectra of the highest resolution are of limited applicability in many important astrophysical problems and also perhaps to indicate the value of cost-effective planning of expensive instrumentation in general.

In addition to providing useful data through measurements of Doppler shifts and line profiles, a prime aim of high resolution spectrometry is the inference of source structure in terms of

temperature distribution of thermal particles³⁻⁶ or energy distribution of non-thermal particles⁶. These distributions are fundamental to our understanding of source mechanisms since they define both the total energy requirements⁷ and the role of energy transfer mechanisms such as conduction⁸. In this respect the X-ray spectrum problem is equivalent to optical/ultra-violet spectral studies of the solar (and stellar) chromosphere-corona structure. The thermal problem is that of the emission from an optically thin non-isothermal atmosphere and may be expressed as^{3,5,7,8}

$$I(\epsilon) = \int_T \xi(T) F(\epsilon, T) dT \quad (1)$$

where $I(\epsilon)$ is the X-ray flux at energy ϵ , $F(\epsilon, T)$ is the emission function at a temperature T per unit emission measure of a plasma of cosmic abundances⁴, and $\xi(T)$ is the emission measure of the source, differential in temperature and normalised for the source distance.

We emphasise at the outset that this equation, and its limitations discussed below, is common to spectrometry of all plasma—solar, cosmic and laboratory.

Mathematically we have to find $\xi(T)$ from $I(\epsilon)$ by inversion of equation (1) which is a Fredholm integral equation of the first kind. Such problems are mathematically ill posed¹⁰. This is distressing since it means that very small changes in $I(\epsilon)$ produce very large changes in ξ . Alternatively, a vast range of ξ 's is capable of reproducing the spectrum within arbitrarily small observational errors.

In practice of course, $I(\epsilon)$ can only be specified at a finite number of points, say n lines or continuum intervals. Given the data vector $Y = \{I(\epsilon_i)\}$ the simplest approach then seeks to find a discrete approximation X to $\xi(T)$ which satisfies

$$AX = Y \quad (2)$$

with $X = \{\xi(T_j) \Delta T_j\}$ where the T_j ($j = 1-n$) are a suitably chosen temperature grid and $A = \{F(\epsilon_i, T_j)\}$ is the matrix representation of the emission function. The required solution can be found by direct inversion

$$X = A^{-1}Y \quad (3)$$

To illustrate the practicalities of this apparently simple situation we now consider the analysis of solar active regions (AR). Attempts to use equation (3) directly for AR analysis using five or more X-ray resonance lines have run several authors^{11,13} into difficulties with nonsensical solutions, that is negative emission measures. Disregarding this salutary warning sign, 'analyses' have been tried to elude the inversion problem by fitting a trial function for X to the data. In all published AR (and solar flare) models^{11,13-17} the fitting function has been chosen purely for expediency, without any physical foundation. In addition, the results are invariably published without proper error analysis and without due consideration of the uniqueness of the model. We now examine these points in detail for a typical active region computation.

Figure 1(a) shows an AR model of the best-fit type¹¹. (This might equally be a cosmic X-ray source model (ref. 12). Using equation (2) we evaluate the fluxes, Y , to be expected in the 5 lines on which this model was based. To test the accuracy (stability) with which X can be derived from these fluxes we perturb the elements of Y with up to 5% random errors so generating a new set \hat{Y} of fluxes, and reinvert by equation (3) to obtain the corresponding new derived structure $\hat{X} = A^{-1}\hat{Y}$. Figure 1b-d show three random realisations of this procedure, and it can be seen that none of these remotely resembles the true structure Fig. 1a. The essential fact is, however, that any of the structures in Fig. 1 (and innumerable others) would reproduce the observed X-ray spectrum to better

than 5%—well within the data accuracy. Had models^{11,13} been published with such an error analysis it would have been immediately clear that their 'best fit' structures were so non-unique as to be physically useless. It follows likewise that models based on minimum χ^2 or other fitting procedures with totally empirical fitting functions containing several adjustable parameters¹⁴⁻¹⁷, are physically meaningless, since an extremely wide range of functions would yield an excellent fit to the data.

Physically this analysis means that the emitted spectrum has a very low sensitivity to any features of source temperature structure other than its crudest nature—for example mean

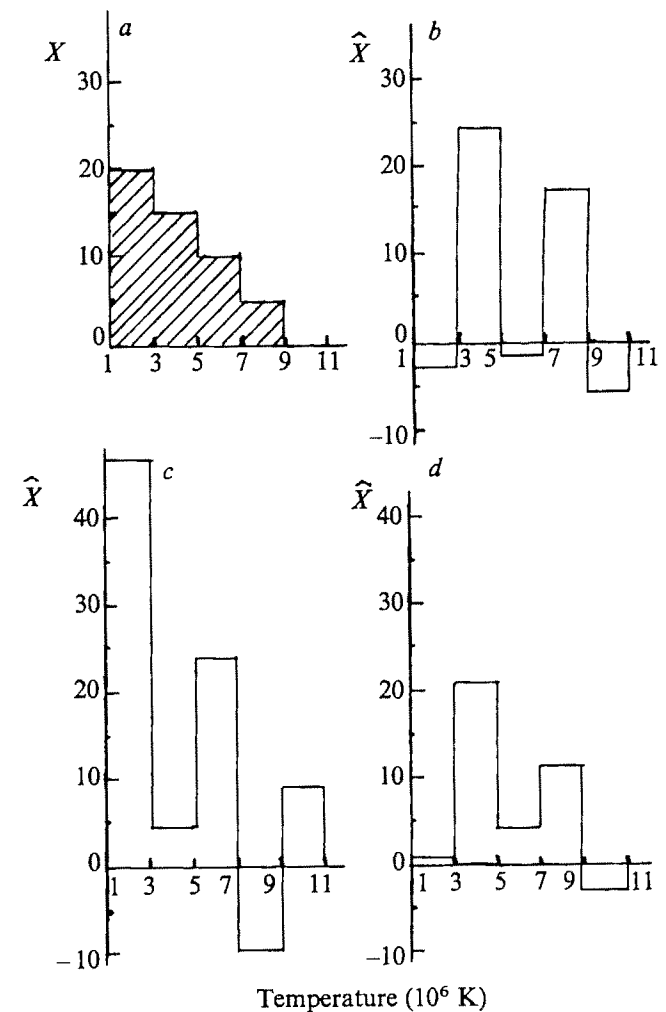


Fig. 1 a, A model (discretised) source temperature structure $X(T)$ which has a spectrum comparable to that observed in ref. 11 and satisfying the constraints they put on $X(T)$. b, c, d, Random realisations of the solutions X obtained on randomly-perturbing the spectral data by up to 5%.

temperature and variance. Theoretically, this result could have been anticipated long before the launch of any instruments. The essence of the problem is that the kernel function F in equation (1) is very flat in temperature (for both lines and continuum) which always leads to severe instability in equations of this type. Alternatively, in matrix form, equation (2), it is readily shown⁸ that errors in \hat{Y} can be magnified in the solution \hat{X} by factors up to η , the condition number of A . (For infinite temperature sensitivity in F , A is diagonal and $\eta = 1$, while for zero sensitivity η is infinite.) For the 5 line analysis quoted above we found $\eta \approx 30$ which explains the nonsensical solutions in Fig. 1 since the errors in \hat{X} can be as large as $30 \times 5\% \approx 150\%$. It should be emphasised that this difficulty cannot be overcome merely by choice of different spectrum lines since all line responses, being governed by excitation from the

Maxwellian electron distribution, contain the factor $\exp(-\varepsilon/kT)$ which is the root of the ill-conditioning of equation (1).

Although the stability problem discussed above is well recognised in other branches of physical science^{18,19} it has so far been neglected in the field of X-ray astronomy. Here, however, the inversion problem is particularly important since knowledge of plasma temperature structure has a direct bearing on other applications of X-ray spectra. For example, computations of element abundance (and also oscillator strengths) are always based on some assumption relating the temperature structure of the source to the temperature sensitivity of lines¹⁷. Thus such methods cannot be decoupled from the difficulties presented here.

In conclusion we emphasise that these snags are not restricted to the inference of thermal structure for solar and cosmic plasmas; they also arise in non-thermal source analysis²⁰. While we agree that limited information on source structures can be obtained from spectral observations, our analysis indicates that such rudimentary information may well be obtained with instrumentation of much lower resolution than is currently available¹⁸. It is vital therefore, in assessing the cost-effectiveness of further X-ray instrumentation, to base future experiments on sound analysis of what is scientifically possible rather than on what is technologically feasible. We therefore recommend a much greater degree of theoretical sophistication in planning satellite experiments since the problem of ill-conditioning, and hence the potential ill-conception of experiments, is indeed universal in physical modelling from data.

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Periodic behaviour of the X-ray flux from the region near 3U1727-33

THE MSSL 2.5–7.5 keV X-ray detector onboard Copernicus¹ was pointed in the vicinity of 3U1727–33 for a total of ~6 h on April 14, 1973 (ref. 2). The $2.5^\circ \times 3.5^\circ$ f.w.h.m. field of view of the detector included the positions of MXB1730–335 (ref. 3), MXB1728–34 (ref. 4) and 3U1727–33 (ref. 5). A new position for 3U1727–33 has been communicated by Saulson and Forman (unpublished). The revised error box is smaller than the one reported in ref. 5 and includes the position of MXB1728–34. The background subtracted count rate observed by Copernicus has been plotted in Fig. 1. Each bin represents one 63-s accumulation followed by 24 s of dead time. The gaps in the data are caused by the occultation by the Earth of the source and passage of the satellite through high back-

ground regions. It is clear from Fig. 1 that the flux is modulated throughout with a period of ~8 min. Using the power spectrum analysis technique described in ref. 6, we find a period of 7.90 ± 0.03 min, with a probability that this could arise from random noise of 10^{-17} and an average modulation amplitude of $23 \pm 5\%$ of the mean flux. Because of the finite sampling time (86.509 s) we cannot exclude the possibility that we are observing an alias of a modulation shorter than the Nyquist frequency of the data. Table 1 gives the first four periods which alias to 7.9 min,

Table 1 Possible aliases to 7.90 min

min	s	Attenuation
7.90 ± 0.05	474.0 ± 3.0	0.97
1.764 ± 0.003	105.84 ± 0.18	0.52
1.219 ± 0.002	73.14 ± 0.12	0.17
0.7933 ± 0.0005	47.598 ± 0.030	0.20
0.6606 ± 0.0004	39.637 ± 0.021	0.20

along with the expected attenuation for a sinusoidal modulation. The mean flux was $\sim 2.5 \times 10^{-8}$ erg cm⁻² s⁻¹, at least two to three times greater than that seen from 3U1727–33 by Uhuru⁶.

The Ariel V experiment C detector⁷ observed the same region of sky for 3 d beginning on March 8, 1976, and again for one day on March 24, 1976 (ref. 8). The experiment C field of view is circular with a f.w.h.m. of 5° , and contained within it were all the sources observed by Copernicus, plus MX1716–30 (ref. 9).

Data with 0.5, 20 and 32 s resolution were obtained in the 2–7 keV range⁸. Both observations have been subjected to a power spectrum analysis and no significant periodicities were detected in the range 45 min to 40 s, with an upper limit of 4% of the mean flux. We then repeated the analysis for each individual orbit of data. In only one orbit (7784, with a time resolution of 20 s) was a significant periodicity detected; this period was 73.1 ± 1.4 s with a probability of random occurrence of 10^{-13} . We note that this period is close to one of the possible aliased periods in the 1973 Copernicus data (Table 1). The mean amplitude of the modulation during this orbit was $14 \pm 3\%$ of the mean flux. Figure 2 illustrates the data of both this orbit and one

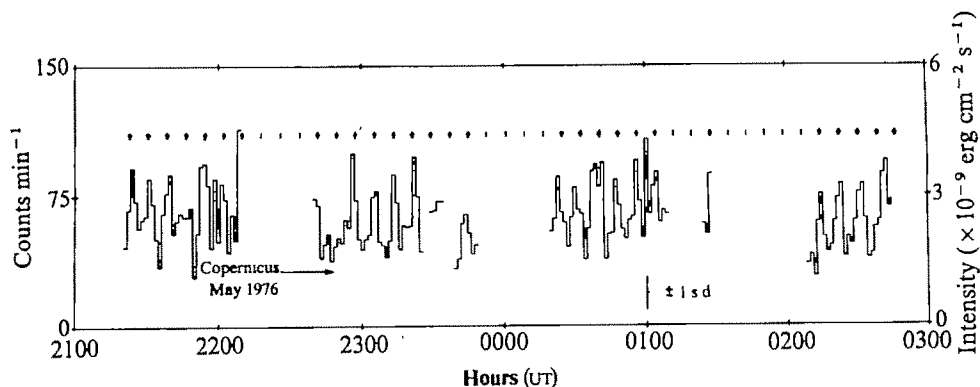
Table 2 Power law fit: $I(E) = CE^{-\alpha} \exp(-\sigma N_H)$

Ariel V experiment C (2.1–20.3 keV)			
Start time day, March 1976	C	α	$N_H \times 10^{22}$
8.570	2.35 ± 0.20	2.05 ± 0.07	5.34 ± 0.02
8.642	2.29 ± 0.18	2.01 ± 0.07	5.33 ± 0.02
9.558	2.38 ± 0.17	2.05 ± 0.06	5.32 ± 0.02
9.629	2.50 ± 0.22	2.07 ± 0.07	5.33 ± 0.02
11.195	2.62 ± 0.22	2.10 ± 0.07	5.33 ± 0.02
11.252	2.52 ± 0.22	2.06 ± 0.08	5.33 ± 0.02
11.335	2.72 ± 0.24	2.11 ± 0.08	5.36 ± 0.02
Copernicus (2.8–8.7 keV)			
May 1976			
16.3	3.5 ± 1.8	2.7 ± 0.5	12 ± 6

preceding it. For the orbit where a modulation was detected the predicted times of maximum flux are indicated. Upper limits on a 73-s modulation obtained for the other 20 s and also the 0.5-s orbits were typically a factor of 3 less than the amplitude of the modulation detected. The mean count rate of each orbit (a flux of $\sim 2.2 \times 10^{-9}$ erg cm⁻² s⁻¹) did not vary by more than ~8%, in particular there was no significant difference between the mean count of the orbit 7784 and those close to it.

Spectral data were obtained on several occasions throughout the first Ariel observation with a time resolution of ~40 min and the results are presented in Table 2. These represent the background subtracted average of all the

Fig. 1 The count rate and flux seen in the April 14–15, 1973 Copernicus observation. The mean intensity recorded by Copernicus in May 1976 is indicated.



sources in the field of view. There is no evidence for any significant spectral variability.

A further half day observation of this region was made by Copernicus on May 16, 1976. The mean count rate observed was ~ 30 counts min^{-1} ($\sim 1.3 \times 10^{-9}$ erg cm^{-2} s^{-1}). The data as a whole show no significant modulation in the

least 50 pulses. As for the first case, there is the possibility that a repetition of the 73-s period has occurred after 3 yr. If this is so, then the modes of oscillation must be limited in number, otherwise the probability of the reappearance of a periodicity would be negligible.

We thank C. Chevalier and S. A. Ilovaisky for discussions,

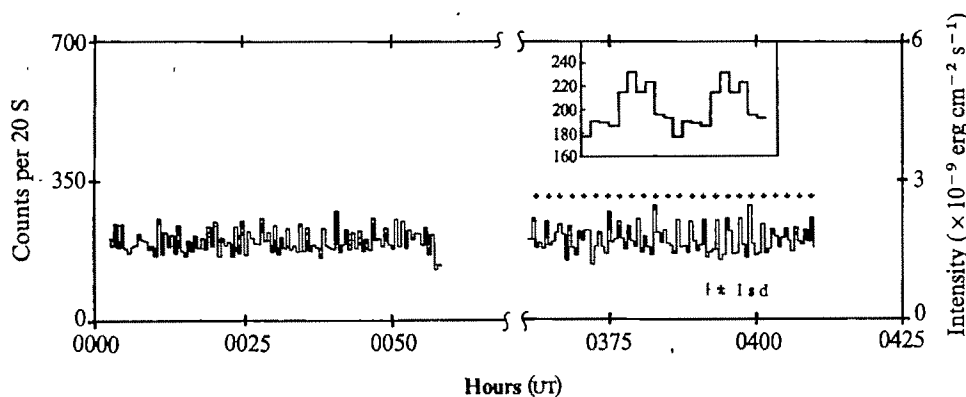


Fig. 2 The background subtracted count rate and flux observed by Ariel V experiment C during two successive orbits 7782 and 7784, in March 1976. For ease of comparison the flux axes have been set to the same as Fig. 1. Also shown are all the data from orbit 7784 folded about a period of 73.14 s and repeated once for clarity.

period 40 min–40 s with an upper limit of 12% of the mean flux. If, however, a transient periodicity such as that seen by Ariel V had been present it would not have been detected because of the poorer statistical significance of the data. Spectral information was obtained with a 6-channel PHA and the spectral parameters obtained are shown in Table 2.

There are two possible interpretations of the data:

(1) The 1973 Copernicus periodicity originated from a regularly pulsating source similar to those reported in ref. 6. Since a similar modulation was not re-observed in 1976 this suggests that either the periodicity originated from a transient source similar to that seen by Ives *et al.*¹⁰, or else the amplitude of the modulation is variable. In the latter case the 73-s periodicity seen in March 1976 could be the fundamental period of the pulsator, or its apparent relationship to the 7.9 min period is coincidental.

(2) The modulation seen in 1973 resulted from a periodic sequence of bursts originating from a burst source in the field of view. The period between bursts would be either 7.9 min or any of its aliases (Table 1). We note that such sequences of bursts have been seen from several burst sources on various time scales (refs 4, 11). In particular MXB1730–335 shows quasi-periodic bursts trains with characteristic time scales between 15 and 35 s (refs 5, 8). To explain the 1973 data we require that bursts trains can be stable for at

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Spiral structure as an explanation for the asymmetric brightness of Saturn's A ring

REITSEMA *et al.*¹ and Lumme and Irvine² have recently confirmed earlier observations which suggested that the A ring of Saturn is fainter in the quadrants following conjunctions of the particles with the Earth-Saturn line and brighter in the quadrants preceding conjunctions. Surprisingly, no intrinsic azimuthal brightness variation is found in the B ring. The brightness variation has been linked to the presence of synchronously rotating particles in the A ring, the effect caused by either a systematic variation in albedo over their surfaces or variations in their geometric projections. Of these two possibilities, the latter seems more promising since the asymmetry of the brightness variation with respect to the Earth-Saturn line is difficult to account for by means of an albedo variation. Furthermore, an albedo variation would yield the same brightness pattern for the B ring if it contained synchronously rotating particles. In this paper, however, we propose yet another mechanism to explain the phenomenon—spiral wakes in the A ring.

The asymmetry would arise in a ring of synchronously rotating, irregularly shaped, particles if there was a statistical tendency for the long axes of the particles to be inclined in the trailing sense with respect to the differential rotation. Such an orientation of the axes of the particles could arise naturally as the result of interparticle collisions and the shear from differential rotation. What is required is that the collision frequency be comparable to the libration frequency of the long axes of the particles (which is $\sim \Omega$, the orbital frequency, for irregularly shaped particles), and that the orbits of the particles deviate from circles by no more than a few times the particle radii a . The first requirement is probably met in Saturn's A and B rings, since their normal optical depths τ_1 are ~ 1 . It is the second requirement that seems unlikely to be satisfied and to which we now turn our attention.

The brightness surge of Saturn's rings as opposition is approached is most plausibly interpreted as the consequence of shadowing in rings that are many-particle radii thick. Best current estimates for the volume density give $D \sim 10^{-3}$ (ref. 3). D is simply related to the vertical thickness of the rings by $D \sim a\tau_1/h$. The random velocity of the particles $v \sim \Omega h$. These relationships imply $v \sim a\Omega\tau_1/D \sim 10^3 a\Omega$ for $D \sim 10^{-3}$ and $\tau_1 \sim 1$. Collisions will produce approximate equipartition between random and rotational kinetic energies, and thus we conclude that the particles in Saturn's rings should have spin periods that are $\sim 10^{-3}$ times their orbital periods. Of course, this conclusion applies in an average sense to the particles that scatter the sunlight. The angular spin velocities ω of individual particles should vary as $(\omega - \Omega) \propto a^{-3/2}$. Thus, particles whose radii are larger than average (the average radius \bar{a} clearly being determined by weighting with respect to surface area) by a factor equal to or greater than

$$\frac{a_1}{\bar{a}} \sim 2^{1/5} \left[\frac{\tau_1}{D} \right]^{2/5}$$

would have $|\omega - \Omega|/\Omega < 1$. A significant fraction of such large particles would be at least temporarily trapped in synchronous rotation if they were irregular in shape. The azimuthal brightness variation might be accounted for if large particles, $a > a_1$, scattered a significant fraction ($\gtrsim 10\%$) of the sunlight incident on the A ring. There is, however, a more attractive mechanism which we shall now describe that is capable of producing the observed effect.

Large particles, whose surface escape velocities are $> v$, will force intense trailing density wakes. Such wakes have been studied previously in connection with spiral structure in galaxies⁴. The detailed mechanics of the formation of wakes in a ring composed of inelastically colliding particles is certain to

be complicated. On the basis of the study cited above, however, there is no doubt that in differentially rotating media these wakes are always oriented in the trailing sense! The dimensions of the trailing density concentrations will range up to a few times the ring thickness h . Since $h \lesssim 4$ km (ref. 3), these density wakes are much too small to be resolved from the Earth but might be resolved by the Mariner Jupiter/Saturn spacecraft due to fly by the planet in 1980. The density wakes, if optically thick, will scatter sunlight in a manner that mimics scattering by synchronously rotating, irregular particles whose long axes are inclined in a trailing sense. The particles in a wake would, however, be only transient neighbours and in no way would a particle and its wake constitute a rigidly rotating entity.

Particles whose surface escape velocities are $\sim v$ have radii $a_2 \sim v/(8G\rho)^{1/2}$. Using the relationships developed previously, we find

$$\frac{a_2}{\bar{a}} \sim \frac{\Omega}{2(2G\rho)^{1/2}} \left(\frac{\tau_1}{D} \right)$$

This expression is to be compared with the expression for a_1/\bar{a} derived earlier. It is easily shown that

$$\frac{a_2}{a_1} \sim 2^{-7/10} \frac{\rho_s}{\rho} \left(\frac{R_s}{r} \right)^3 \left(\frac{\tau_1}{D} \right)^{3/5}$$

where $\rho_s = 0.7$ g cm⁻³ and $R_s = 6 \times 10^9$ cm are the mean density and the mean radius of Saturn. Taking $\rho = 1$ g cm⁻³, $r = 1.3 \times 10^{10}$ cm, $\tau_1 = 0.5$ and $D = 10^{-3}$, which are appropriate parameters for ice particles near the centre of the A ring, we obtain $a_2/a_1 \sim 0.4$. Thus, particles that are large enough to rotate synchronously will also force dense trailing wakes.

To this point, our discussion has been based on the assumption that the opposition effect implies $D \sim 10^{-3}$. We stress that if this assumption were incorrect and $D \gg 10^{-3}$, our conclusion, that density fluctuations rather than synchronously rotating particles are responsible for the azimuthal brightness variation, would be strengthened (note $a_2/a_1 \propto D^{-3/5}$). Furthermore, if the mean mass density in the rings were as high as 0.1 g cm⁻³, the rings would be on the brink of gravitational instability^{5,6}. In this case, collective gravitational effects would greatly enhance the strength of trailing density perturbations⁴.

The absence of an azimuthal brightness variation in the B ring must still be explained. Two possibilities immediately come to mind. First, the higher optical depth of the B ring would make its brightness less sensitive to either the geometrical orientations or the spatial correlations of the individual particles. Second, both mechanisms for producing the azimuthal brightness variation require the rings to contain particles whose radii are considerably larger than average. Small particles are more likely to gravitationally aggregate and form large particles in the A ring than in the B ring. In this context, we note that the formal Roche limit for particles with $\rho = 1$ g cm⁻³ occurs at $r = 1.3 \times 10^{10}$ cm, near the centre of the A ring.

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Circumstellar acetylene in the infrared spectrum of IRC +10° 216

A THICK, expanding envelope of dust and gas surrounds the unusual object IRC +10° 216. Microwave studies have identified numerous molecular constituents of the envelope¹, and the thermal infrared spectrum indicates the probable presence of both silicon carbide and graphite in the form of dust grains (ref. 2, and M. P. Campbell, *et al.*, unpublished). We report here the detection of acetylene, C₂H₂, in the infrared spectrum. Although acetylene is expected to be an important constituent of some stellar atmospheres, and possibly of interstellar molecular clouds, this appears to represent the first positive detection of C₂H₂ outside the solar system.

A Fourier transform spectrometer at the coudé focus of

the Mayall 4-m telescope at Kitt Peak was used to obtain a spectrum of the region 4,000–5,000 cm⁻¹ (2.0–2.5 μm). This was a daytime observation, and a solar reference spectrum was recorded with a small heliostat shortly before and at similar zenith distance. A very small portion of these spectra are shown in Fig. 1. The +10° 216 spectrum contains a resolved, red degraded feature at 4,091 cm⁻¹ which we attribute to the ν₁+ν₅ band of acetylene³. Only the Q branch is detectable. Lines Q(1) to Q(7) fall in an interval of only 0.12 cm⁻¹. In the P and R branches, the lines are too widely separated to yield distinctive features.

The strength of the feature drops sharply near J=16, suggesting a temperature of 200–300 K, so the absorption is most probably associated with the 2'' diameter circumstellar shell⁴. Adopting a temperature of 250 K, the strongest acetylene absorption would be expected to occur at 4,090.98 cm⁻¹. The observed maximum absorption, corrected for the Doppler shift appropriate for the 2'' shell (ref. 5 and D. N. B. Hall, *et al.*, unpublished), falls at 4,090.97 ± 0.03 cm⁻¹, indicating the internal consistency of our interpretation. From published spectra^{6,7} and intensity studies^{8,9} we estimate a ν₁+ν₅ Q branch intensity of 0.2 cm⁻² atm⁻¹. The observed equivalent width of 0.2 cm⁻¹ then requires a column abundance ~1 cm atm, or 3 × 10¹⁹ mol cm⁻². For a 2'' diameter shell and a distance of 200 pc (D. N. B. Hall, *et al.*, unpublished) this corresponds to a total mass of ~10⁻⁴ M_⊙.

Of the other important C₂H₂ bands only the 3.04-μm band⁹ should definitely be detectable in existing +10° 216 spectra. A previously unidentified band in fact occurs in many carbon stars¹⁰ at the correct position. The strength of this band in +10° 216 (~90 cm⁻¹) could reasonably arise from 1 cm atm of acetylene, and we suggest that acetylene does in fact contribute part or all of this feature. Absorption in the 3 μm region from other hydrocarbons is, however, likely in carbon-star photospheres, and cannot be ruled out in the +10° 216 shell.

Except for the 2–0 band of CO (D. N. B. Hall, *et al.*, unpublished) no other lines were found in the 4,000–5,000 cm⁻¹ region to a limiting equivalent width of ~0.05 cm⁻¹. From laboratory spectra¹¹ of the 2.32 and 2.37 μm bands of methane, we estimate a CH₄ abundance <0.03 cm atm, or 7 × 10¹⁷ mol cm⁻².

IRC +10° 216 is apparently a site of continuing production and ejection of grains and molecules. In view of the serious difficulties encountered in developing a satisfactory understanding of these processes, +10° 216 deserves detailed and continuing study. In particular, two important questions may be resolved by continued investigation: first, is grain formation a cause or an effect of mass ejection?; second, do molecular abundances 'freeze out' on ejection? Our results for C₂H₂ and CH₄ (which are not detectable in the microwave region because of lack of pure rotational dipole transitions) are particularly relevant to the second question. The large C₂H₂ abundance, comparable to the CO abundance (ref. 5 and D. N. B. Hall, *et al.*, unpublished), is consistent with computations for cool, carbon rich stellar photospheres¹². The predominance of C₂H₂ over CH₄ is also expected. If the photosphere were ejected in such conditions that it cooled while maintaining molecular equilibrium, however, C₂H₂ should be strongly depleted¹³. Morris¹ has compared relative abundances of SiS, SiO, CS and HC₃N with recent molecular equilibrium calculations¹⁴. While inconclusive, his results did not rule out 'freezing' of photospheric abundances. Additional spectroscopic study should provide crucial relative abundances (or upper limits) for C₂, CN, HCN, and possibly others, permitting a more conclusive analysis of the evolution of the molecular abundances during ejection.

A number of infrared objects are known which appear to be similar to +10° 216 (ref. 10). In addition, certain bright carbon stars show a 10-μm flux excess, attributed to circum-

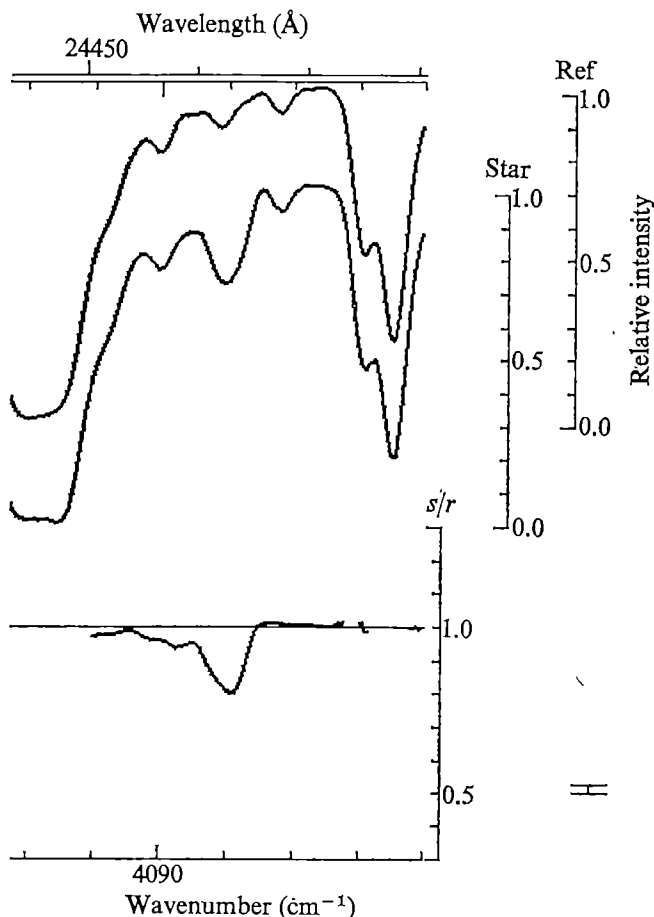


Fig. 1 The spectrum of IRC +10° 216. Above, a comparison solar spectrum (dominated by terrestrial absorption); middle, the sum of two +10° 216 spectra; below, the ratio +10° 216/sun. A 2σ error bar, computed from the difference of the two +10° 216 spectra, is shown at the lower right. The resolution is 0.25 cm⁻¹, and the FWHM of the instrumental line profile is 0.30 cm⁻¹. The abscissa scale at the bottom is in units of 1 cm⁻¹/tic (vacuum) increasing to the right; at the top, units are 10 Å/tic (STP) increasing to the left. The Doppler shift of +10° 216 at the time of observation was negligible for purposes of this figure, but has been accounted for in the analysis. In the ratio +10° 216/sun the small peak at 4,090.52 cm⁻¹ is an artefact arising from a weak absorption line in the solar spectrum.

stellar dust¹⁸. We have obtained a spectrum of one of the latter, V Cygni. In this star the 4,091 cm⁻¹ band appears prominently, stronger than in +10° 216, and apparently originating in a circumstellar region of somewhat higher temperature. It is possible that the circumstellar acetylene and dust grains are mixed at a common kinetic temperature. If so, progress may be possible in the difficult problems of determining grain emissivity and particle size distribution.

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Time-varying Newtonian gravity and universal motion

A TIME-dependent gravitational constant $G(t)$ was proposed by Dirac¹ in 1937, and there is some limited evidence for it^{2–4}. It can be incorporated in relativistic theories of gravitation (see refs 5–7), but the resulting equations are liable to be cumbersome, and it is uncertain which theory should be used. Attempts to consider this problem in a Newtonian framework^{3,4,8–10} have been based on the amended Newtonian force law

$$\mathbf{F} = -G(t) \frac{m_1 m_2 \mathbf{r}}{r^3} \quad (1)$$

We show here that the assumption of energy conservation (as discussed below) leads to a more substantial change in the force law.

That equation (1) implies violation of energy conservation for particle interactions can be seen as follows. One particle, shaped like a doughnut, moves from infinity directly towards a second particle; the particles move through each other to infinity. If $G(t)$ is decreasing, then, for the same distance of separation, the force of attraction whilst the particles are approaching is greater than the force whilst the particles are moving apart. The final relative velocity for large separation is greater than the initial relative velocity. Thus the final kinetic energy is greater than the initial kinetic energy. Since the potential energy is zero for large separation, energy conservation for interacting particles has been violated; the energy of the gravitational field is

not involved. Energy conservation for interacting static bodies would of course also be violated.

We see that energy conservation and equation (1) cannot both hold in the generalised Newtonian theory. In the past the failure of the energy law appears to have been ignored, and equation (1) has been maintained, presumably as the more physically important of the two laws. In this note an alternative path is explored: a new force law will be obtained from energy conservation, which, together with expressions for kinetic and potential energy, will be assumed.

We consider a finite set of discrete particles with masses m_α ($\alpha = 1, \dots, N$). We shall take the potential energy, V , of the system in the general form

$$V = \frac{1}{2} \sum_{\substack{\alpha, \beta=1 \\ \alpha \neq \beta}}^N m_\alpha m_\beta v(r_{\alpha\beta}, t) \quad (2)$$

Referring to a chosen inertial frame, \mathbf{x}_α is the position vector of the particle α ; $\mathbf{r}_{\alpha\beta} = \mathbf{x}_\alpha - \mathbf{x}_\beta$; $r_{\alpha\beta} = |\mathbf{r}_{\alpha\beta}|$; and the function

$$v(r_{\alpha\beta}, t) = \frac{-G(t)}{r_{\alpha\beta}} \quad (3)$$

would be the obvious choice. The kinetic energy of the system has the usual form, and the energy conservation law is

$$\sum_{\alpha=1}^N \frac{1}{2} m_\alpha \sum_{l=1}^3 \dot{x}_{\alpha l}^2 + \frac{1}{2} \sum_{\substack{\alpha, \beta=1 \\ \alpha \neq \beta}}^N m_\alpha m_\beta v(r_{\alpha\beta}, t) = E \quad (4)$$

where E is a constant. Total differentiation with respect to time of equation (4) leads, after some algebra, to

$$0 = \sum_{\alpha=1}^N m_\alpha \sum_{l=1}^3 x_{\alpha l} \left(\dot{x}_{\alpha l} + \sum_{\substack{\beta=1 \\ \beta \neq \alpha}}^N m_\beta \left[\frac{\partial v}{\partial r_{\alpha\beta l}} + \frac{\dot{r}_{\alpha\beta l}}{V_{\alpha\beta}} \frac{\partial v}{\partial t} \right] \right) \quad (5)$$

where $V_{\alpha\beta} = |\dot{\mathbf{r}}_{\alpha\beta}|$. Equation (5) suggests, and is consistent with, the force law ($i = 1, 2, 3$)

$$x_{\alpha i} = - \sum_{\substack{\beta=1 \\ \beta \neq \alpha}}^N m_\beta \left[\frac{\partial v}{\partial r_{\alpha\beta i}} + \frac{\dot{r}_{\alpha\beta i}}{V_{\alpha\beta}} \frac{\partial v}{\partial t} \right] \quad (6)$$

A formal approach to the preceding paragraph is as follows. Newton's three laws of motion apply, and the particle interaction force is assumed to be given by equation (6). Equation (5) follows immediately, and may be integrated to show that equation (4) is a constant of the motion. Because of the form of equation (4), it is physically appealing to identify this constant as the energy.

The ratio, X , of the second term to the first term in equation (6), subject to equation (3), is estimated for a particle moving with speed V (cm s⁻¹) on the surface of the Earth (taken as a sphere of radius r)

$$|X| \simeq \left| \frac{\dot{G}}{G} \right| \frac{r}{V} < \frac{10^{-8}}{V}$$

where $|\dot{G}/G| < 10^{-10} \text{ yr}^{-1}$ has been used. For two atoms at a distance apart, a , $\sim 10^{-8} \text{ cm}$ in a solid at absolute temperature T , taking $V \simeq (kT/m)^{1/2}$, one finds similarly

$$|X| \approx \left(\frac{m}{kT} \right)^{1/2} a \left| \frac{\dot{G}}{G} \right| < \frac{10^{-20}}{T^{1/2}}$$

The correction to the second term in equation (6) is thus normally negligible.

Even if equation (3) is not used, the force law, equation (6), has the remarkable implication that if the law of gravitation, equation (2), is time-dependent, then, to avoid infinite forces, no two particles in the Universe may be at relative rest for a non-zero time interval. The subject of statics must be considered as an approximation if the above ideas are accepted, quite apart from arguments based on quantum mechanics. Our principle of universal motion is in agreement with the observed fact that physical systems on all scales are in states of relative motion: there are zero point vibrations in condensed systems; planets orbit stars; galaxies rotate; and the Universe itself is expanding.

In conclusion, we note the following points:

(1) Newtonian cosmology with time-varying gravity has been discussed previously using an energy conservation law^{11,12}. The application of the force law, equation (6), to cosmology has been considered¹³, and yields results consistent with the energy approach.

(2) Equation (6) is Machian, in that the force on a particle depends on the velocity of all other particles in the Universe.

(3) Since equation (6) leads to an interparticle force which need not act along the line joining the two particles, angular momentum is not conserved in general.

(4) It would be desirable to find a variational principle leading to equation (6).

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Thermal alteration of young kerogen in relation to petroleum genesis

AVAILABLE evidence indicates that petroleum hydrocarbons can be generated by alteration of kerogens through geothermal heating¹⁻³. In this study we have separated kerogen, humic acid and lipid (benzene-methanol extractable) material from a young marine sediment (Tanner Basin, offshore California) and heated it in sealed glass tubes in a nitrogen atmosphere. Each tube was heated at a single temperature between 150 and 410 °C for a specified time in the range 5–120 h. Gaseous and liquid (benzene-methanol extractable) products generated during the heating, as well as the residual organic material, were characterised by gas-liquid chromatography, elemental analysis, infrared and electron

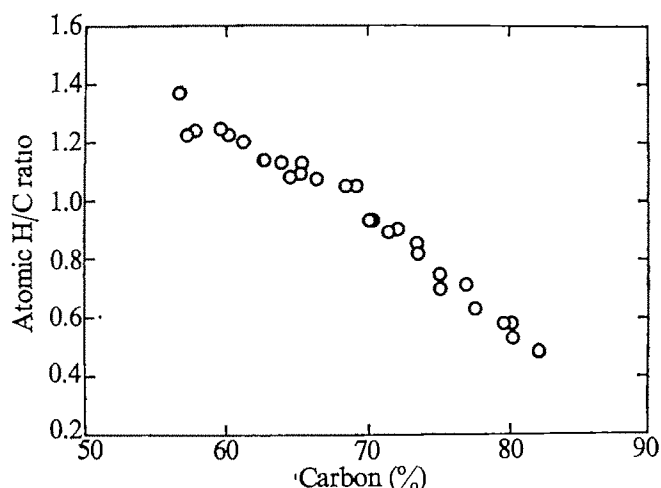


Fig. 1 Changes in H/C ratio and percentage C of kerogen when heated.

spin resonance (ESR) spectroscopies, and X-ray diffraction.

The sediment sample used has approximately 6% (by dry weight) of organic carbon. The lipid, humic acid and kerogen fractions account for 10, 7 and 72% of the total organic matter, respectively. Unheated kerogen contains 56.7% carbon, 6.5% hydrogen, 6.0% nitrogen and 30.8%

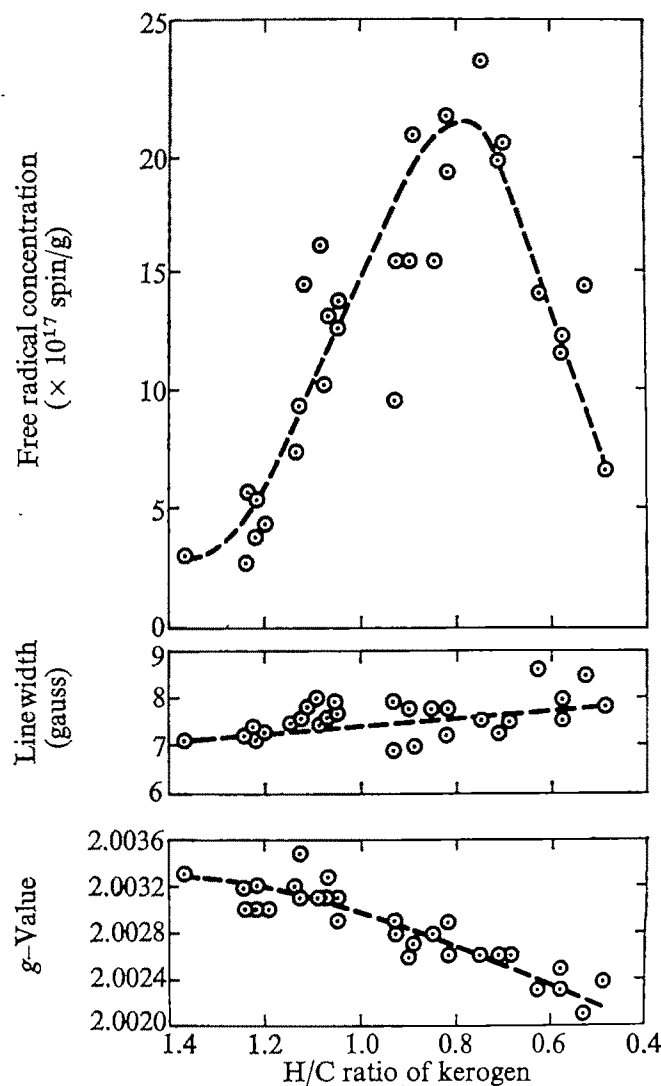


Fig. 2 Relationship between ESR properties of kerogen and its H/C ratio.

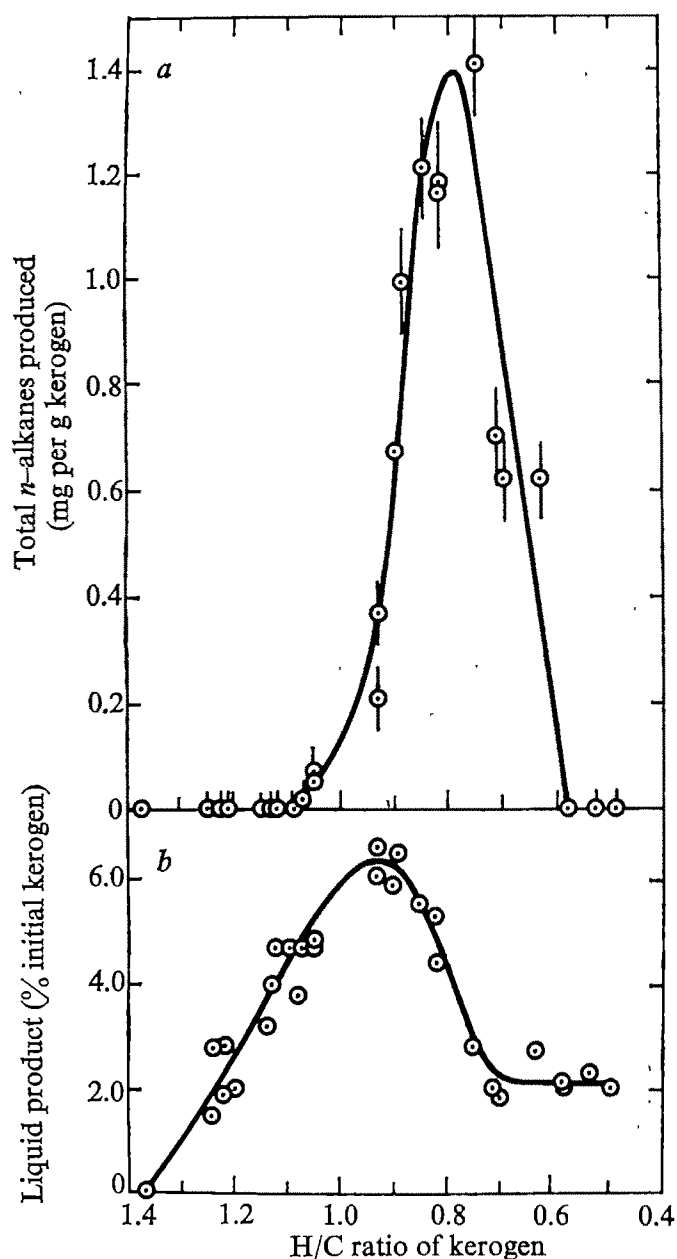


Fig. 3 Total normal alkanes and liquid product generated are plotted against the H/C ratio of kerogen.

oxygen (by difference) on an ash-free basis, and seems to fit into the type III kerogen classification of Tissot *et al.*³.

With increasing time and temperature, between 150 and 410 °C, the carbon content of kerogen increased from 56.7 to 82.1% and its atomic H/C ratio decreased from 1.37 to 0.49 (Fig. 1). Therefore, the values of either percentage C or the H/C ratio of residual kerogen may be used as an index for "level of organic maturation" (LOM) for this type of kerogen. Figure 2 shows plots of H/C ratios of residual kerogen against various parameters derived from ESR spectroscopy. With increasing degree of thermal alteration of kerogen, spin concentration increases, which may be closely associated with bond cleavage and hence, the elimination of functional groups. Below an H/C value of 0.7, the spin concentration decreases again, due to the gradual conversion of kerogen to a graphite-like structure, as verified by X-ray diffraction analysis. The kerogen g-value decreases with increasing degree of thermal alteration. This indicates the change of free radicals from semi-quinone type to radicals of carbon, nitrogen and sulphur due to imperfection in the kerogen structure⁴. Changes in ESR

characteristics on heating follow the same general pattern as those described by Pusey⁴ for kerogen in sedimentary rocks and Van Krevelen⁵ for coals (Fig. 2).

During heating, kerogen produced large amounts of gaseous product, amounting to about 40% of the initial weight at 410 °C. In the initial stages of thermal alteration, only H₂O and CO₂ were evolved. With increasing degree of thermal alteration, hydrogen, methane and other volatile hydrocarbons began to be generated, and their content increased gradually.

In addition, liquid products soluble in organic solvents began to be produced at a low temperature of alteration, reached a maximum at a kerogen H/C ratio of 0.90–0.95, and then decreased as thermal alteration increased. The decrease in the amount of liquid product may be explained by its further decomposition on heating. In Fig. 3a, the relationship between the total (C₁₃–C₄₁) normal alkanes or paraffins generated and H/C of kerogen is recorded. For the Tanner Basin kerogen, paraffins begin to be generated around H/C=1.1, increase sharply, reach a maximum

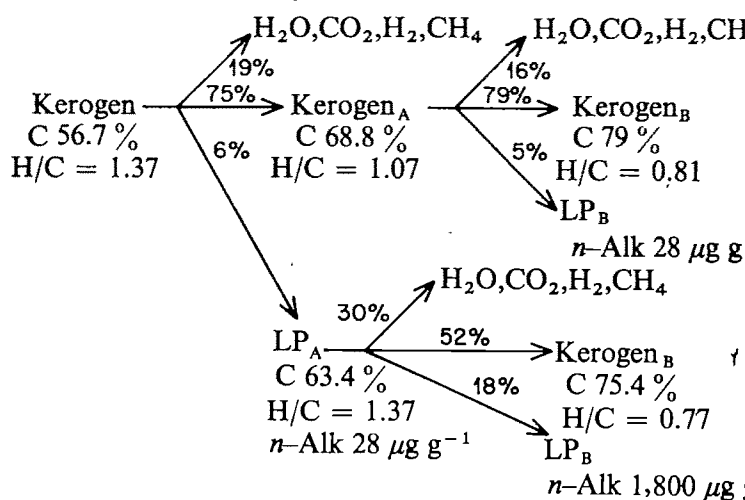


Fig. 4 Generation mechanism of n-alkanes from kerogen on heating.

around H/C=0.8, and then decrease rapidly. The apparent activation energy of the generation of the total n-alkanes was calculated to be 31 kcalorie mol⁻¹, which is lower than that of the breakage of C–C bonds in n-alkanes (58 kcalorie mol⁻¹)⁷. Therefore, generation of n-alkanes from kerogen may be due to the breakage of bonds other than C–C, for example, functional side chains on aromatic or cyclic rings, or compounds containing O, N or S, where the activation energy for bond breakage is lower than for paraffins⁸.

It is quite interesting to note that the H/C ratio of Tanner Basin kerogen at the maximum production of total liquid product (H/C=0.90–0.95; see Fig. 3b) is different from that at the maximum production of n-alkanes (H/C=0.8). This suggests that paraffins may be generated by the subsequent decomposition of the liquid product. A similar conclusion was reached by Tissot's group in the heating experiment of kerogen from a shale⁹. To elucidate further the mechanism of hydrocarbon generation, our kerogen was heated at 260 °C for 18.5 h, the optimum conditions for the generation of liquid product but too low to produce much n-alkanes. By this reaction, liquid product (LP_A) and kerogen (A) were obtained, and then those two fractions were heated again at higher temperature (330 °C, 24 h) to produce paraffins (Fig. 4). Clearly, the liquid product obtained on heating at the lower temperature is more aliphatic (H/C=1.37) than the residual kerogen (A) (H/C=1.07). By the second heating at the higher temperature, the liquid product (LP_A) produced a large amount of normal paraffins, as well as highly aromatic kerogen-like material. Heating the kerogen (A), also pro-

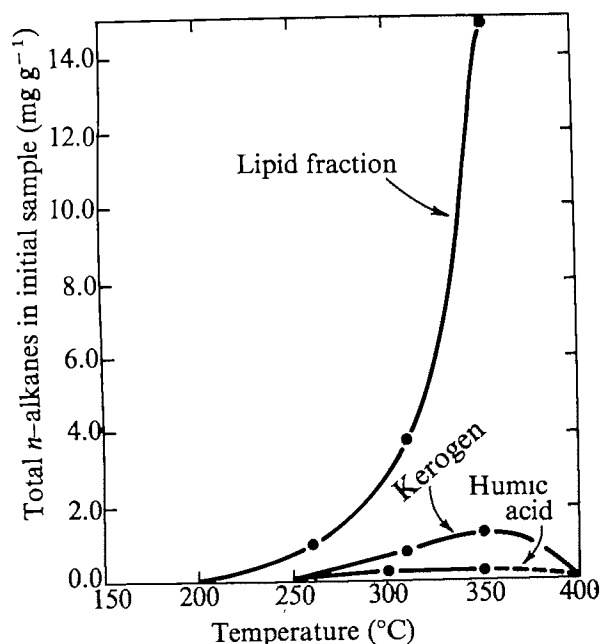


Fig. 5 Changes in the amount of total *n*-alkanes generated from various organic fractions on heating at various temperatures for 24 h.

duced alkanes and highly aromatic kerogen (B), but only about a third of the amount produced from the liquid product (LP_A). These results support the model for the production of normal hydrocarbons proposed by Tissot's group.

Interestingly, the distribution pattern of *n*-alkanes generated from kerogen during the initial stage of thermal alteration, showed an even carbon number predominance (CPI ≈ 0.5). With increasing degree of thermal alteration, the distribution pattern approached that of petroleum hydrocarbons (≈ 1.0).

The results of heating other organic sediment fractions (lipid and humic acid) showed that these fractions also have an ability to produce paraffinic hydrocarbons (Fig. 5). The amounts produced are, however, very different. The contribution of each organic fraction to the total alteration was calculated for the Tanner Basin sediment sample. The lipid fraction accounts for 58%, humic acid 0.4% and kerogen 41% of the total amount of normal hydrocarbons produced.

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Photocell using covalently-bound dyes on semiconductor surfaces

We have developed a new type of electrochemical photocell, using an optically transparent semiconductor electrode whose surface was modified by the chemical binding of sensitising dyes. In spite of the absence of dye in the solution, a photocurrent, as large as those previously observed at the unmodified electrodes in contact with the dye solutions was obtained.

Since the initial report by Fujishima and Honda¹ on a photocell employing a TiO₂ n-type semiconductor as the photoanode to decompose water under illumination by photons of energy greater than the band gap, there has been much research²⁻⁶ on this subject because of its important implications for solar energy conversion and hydrogen production. Photosynthetic light conversion⁷⁻¹⁰ in green plants has also been studied. From the commercial use of electrochemical photocells as solar energy conversion devices, the photoelectrodes should be stable on illumination in aqueous solutions. TiO₂ and SnO₂ are the two candidates that have been so far found to be inert¹¹⁻¹³. The band gaps of TiO₂ and SnO₂ are, however 3.0 eV (ref. 14) and 3.5 eV (ref. 15) respectively which correspond to photon wavelengths of 415 and 355 nm. Solar energy reaching the Earth's surface has a spectrum distribution in longer wavelength¹², and cannot therefore be used. Effective ways of extending the sensitivity of electrochemical photocells to longer wavelengths are therefore strongly desired. A better way to solve the problem would be spectral sensitisation, in analogy with the primary light reaction of photosynthesis, where chlorophyll molecules accept the solar energy. A noticeable anodic current can be seen in electrochemical cells containing an n-type semiconductor anode in contact with certain dye solutions when illuminated with light in the spectral region where the dye absorbs, even though of energy less than the band gap of the semiconductor. This electrochemical spectral sensitisation works for various electrode materials, including TiO₂ (ref. 16) and SnO₂ (ref. 17), and has been mainly studied to elucidate the mechanism of the spectral sensitisation, since Gerischer *et al.*¹⁸ started using ZnO single crystal.

As sensitisers, dyes belonging to the xanthene and the cyanine groups have been widely used. Gerischer and Tributsch¹⁸ observed also an increase in the sensitised photocurrent on adding of reducing agent, such as hydroquinone,

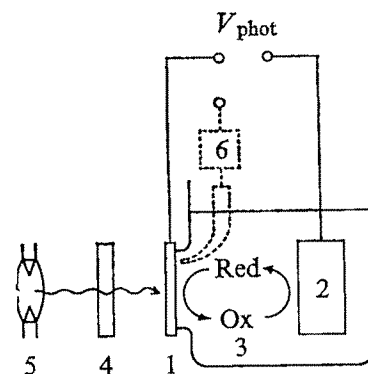
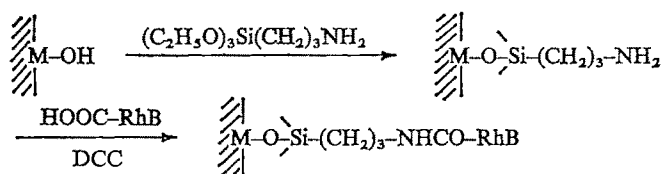


Fig. 1 An electrochemical photocell consisting of an optically transparent semiconductor anode chemically modified with rhodamine B (1) and a Pt cathode (2) in contact with an electrolyte containing a redox system (3). As a light source, a 500-W xenon lamp (5) was used in conjunction with filters and/or a monochromator (4). For measurements of spectral sensitisation (shown in Fig. 2) the electrode was polarised with a conventional potentiostat using a saturated calomel electrode (6) with a Luggin capillary.

Na_2SO_3 , or H_2O_2 , to the dye solution, although the reducing agents show no absorption in the visible region. This phenomenon is termed 'supersensitisation.'

For a real photocell, however, a new problem may arise: most of the solar energy is absorbed ineffectively by the dye molecules in the solution rather than by the adsorbed dye molecules at the electrode-solution interface, though only the excited adsorbed dye molecules can contribute to sensitisation²⁰.

To cope with this, a new type photocell has been developed (Fig. 1). The chemically modified SnO_2 or TiO_2 electrode with rhodamine B as a sensitizer is in contact with the transparent electrolyte solution containing a reducing agent as a supersensitizer. The procedure to modify the electrodes was similar to that previously described²¹, but synthesis of amide-linked rhodamine B by the dehydrative coupling of a carboxyl group of rhodamine B with propylamine modified electrodes was carried out using dicyclohexylcarbodiimide (DCC) as a dehydrating agent because it gives a better yield than the two-step reactions, involving the preparation of acid chloride of rhodamine B using thionyl chloride and the acylation of the propylamine



M = Sn or Ti. HOOC-RhB = rhodamine B

modified electrodes with the acid chloride. The propylamine modified TiO_2 electrodes were prepared in the same manner as the propylamine modified SnO_2 electrodes²¹ by the method developed by Murray *et al.*²². The surface reactions and confirmatory chemical tests were followed by X-ray photoelectron spectroscopy²³.

Since the rhodamine B was covalently bound by silyl ether and amide bonds to the electrode surface, the dye remained almost permanently on the electrode surface, unless the pH value of the electrolyte solution was extremely low or high. Unwanted decomposition of the dye in the course of

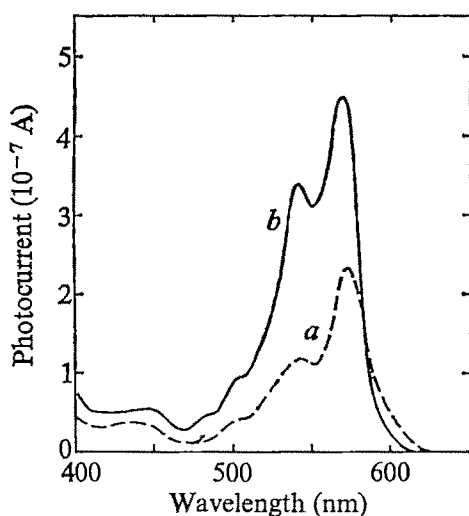


Fig. 2 Spectral dependence of the photocurrent for optically transparent SnO_2 electrodes polarised anodically at + 0.35 V against SCE, and illuminated through the electrode (Fig. 1). *a*, Photocurrent spectrum sensitised by a sub-monolayer of rhodamine B covalently bound to the electrode surface through amide bonding at the chemically modified SnO_2 electrode in contact with a transparent electrolyte (0.2 M Na_2SO_4) containing hydroquinone (5×10^{-3} M) as a supersensitizer. *b*, Photocurrent spectrum at the conventional unmodified SnO_2 electrode in contact with a rhodamine B solution (5×10^{-4} M rhodamine B + 5×10^{-3} M hydroquinone in 0.2 M Na_2SO_4).

the sensitisation process was almost entirely suppressed by the presence of the supersensitizer in the solution²⁴.

A typical sensitised photocurrent spectrum obtained with the modified electrode is shown in Fig. 2, as well as a spectrum obtained with the conventional untreated electrode in contact with rhodamine B solution containing the same amount of supersensitizer. The anodic photocurrents observed at the modified electrode, whose spectral dependence was quite close to that of the dye's absorption spectrum, is the same order of magnitude as those observed at the unmodified electrode, even though there is no dye in the solution. Since a monolayer coverage of the dye absorbs very little light^{24,25}, the modified electrodes are almost indistinguishable to the eye from the unmodified ones, and SnO_2 electrodes are transparent in the visible region. Accordingly, further improvements in efficiency can be easily attained by immersing many modified electrodes in the electrolyte solution in parallel containing colourless supersensitizers, and then by illuminating them perpendicularly.

Perhaps, the illumination of the electrode-electrolyte interface in a multiple internal reflection mode, (ATR, refs 24-27) will also be effective, since the chemically bound dyes on the surface can be excited by the 'evanescent wave', and loss of energy through reflections can be avoided in this mode. Extension of the useful spectral region may be possible by binding various sensitizers.

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Cultural, language and geographical correlates of genetic variability in Andean highland Indians

The genetic structure of primitive human populations has been studied for the purposes of understanding hominid evolution and developing more cogent genetic models. For this it is important not only to demonstrate genetic variability between populations, but also to explore the correlates of such variability. Using data on several Chilean Indian populations I have found that genetic differentiation can best be ascribed to geographical separations. Other correlates, such as culture and language, are of secondary importance when the relationship is studied simultaneously by a stepwise regression technique¹. Although I consider here a particular set of populations, the results obtained

Table 1 Correlation coefficients between genetic, geographical, cultural and linguistic variations among seven Chilean Indian tribes and their association with each tribe's extent of Indian ancestry

	Geographical distance	Cultural dissimilarity index	Linguistic dissimilarity index	Probability of Indian ancestry
Genetic	0.716*	0.775*	0.032	-0.018
Geographical	—	0.901*	0.323	-0.142
Cultural	—	—	0.147	0.016
Linguistic	—	—	—	-0.194

*Significant at 0.01 level.

seem to be fairly generally applicable². I have found an empirical relationship between geographical distance and gene identity for the Andean highland Indians discussed here.

There have already been attempts to relate genetic differentiation within a tribe or between several primitive tribes to linguistic, social, topographical and environmental components of the population²⁻⁸, but the conclusions were not always unequivocal. This is partly because all such correlates are themselves associated with one another, so that a simple analysis of variance as advocated by some researchers⁷, or a simple pairwise correlation⁴ may result in an unwarranted interpretation of the results.

The Chilean Indian tribes considered in this analysis are

organisation of the seven tribes. Another correlate was the linguistic variability which emerged from the nested hierarchical classification of Andean languages¹⁴. From the approximate geographical location of the tribes, the geographical distance between them was computed following standard physical transformation. As all the tribes have intermixed with Caucasoid invaders, the variation in the degree of Caucasoid admixture as determined from the ABO blood group were used to derive the probability of Indian ancestry for any two genes of the tribes.

To determine the nature of interrelations among these measures of variability, I first present the correlation coefficients between them using all the pairwise tribe-comparisons ($C_2=21$, see Table 1). Cultural and

Table 2 Results of a stepwise regression analysis identifying interrelationships between the different variability measures in Chilean Indian tribes

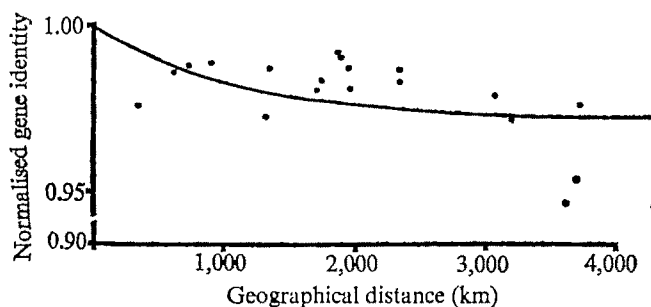
Variables entered	% Variance of genetic distance explained Cumulative	Marginal	F ratio (for each successive step)	Probability
Geography	51.31	51.31	20.02	$P < 0.001$
Culture	60.19	8.88	4.01	$0.05 < P < 0.1$
Language	61.48	1.29	0.57	$0.4 < P < 0.5$
Probability of Indian ancestry	61.52	0.04	0.02	$0.8 < P < 0.9$

the Aymara (17°S, 70°W), Atacameño (23°S, 67°W), Diaguita (22°30'S, 63°42'W), Pehuenche (38°S, 71°30'W), Mapuche (38°S, 72°30'W), Acahuaf (50°S, 75°W) and Yagan (55°36'S, 70°48'W), all so-called 'highland' Andean tribes. Gene frequencies for seven serological markers (ABO, MNSS, Rh, Diego, Duffy, Kell and haptoglobin) common to each population were extracted from the literature⁴⁻¹². To measure the genetic differentiation based on these markers, I computed the standard genetic distances between the different tribal groups, using the methods of Nei¹³.

As a first correlate of genetic differentiation, I constructed a cultural dissimilarity matrix taken directly from work⁴ involving an extensive literature survey of Andean culture. The basic features involved in cultural classification were the mode of subsistence, economics and social

geographical distances are almost linearly related ($r=0.901$). Linguistic dissimilarity was a poor correlate of genetic differentiation ($r=0.0320$, $P>0.85$). When geographical distance is used as the only regressor to explain genetic variabilities in the seven tribes, about 51.3% of the total variance is explained. All the other variables (cultural and linguistic variabilities, and variation in rates of admixture with Caucasians) explain only a marginal amount (10.2%). The result of such a regression analysis is shown in Table 2. This analysis also indicates that of all the variables, only geographical distance seems to be important, explaining the trend in genetic variabilities in these seven Andean populations. This agrees with theoretical predictions¹⁵. The relationship between genetic distance and geographical distance can be studied further by relating the probability of allelism between two homologous genes with the geographical distance by which two groups are separated. The probability of allelism, or the probability of gene identity between two populations, I_r , is given by $I_r = \exp(-D)$, where D is Nei's measure of standard genetic distance¹³. Figure 1 represents the empirical relationship between normalised gene identities (I_r) and geographical distances (r) for the 21 tribe comparisons. The least square fit of $I_r = 0.973 + 0.027 \exp(-r/1,000)$ is statistically satisfactory ($0.05 < P < 0.1$), although there are a few large deviations from expectations towards the tail of the curve.

There is also a high association between cultural and genetic variabilities in the seven tribes (Table 1), but this may be an artefact of the other two significant values in the same table. Such an association is also exhibited through the regression analysis, in the sense that in addition to geographical distance, cultural differences do not contribute significantly to the explanation of variability in genetic

Fig. 1 Empirical relationship between normalised gene identity and geographical distance (km) as studied from seven Chilean Indian tribes.

distances. The high correlation between culture and geography may also be interpreted from historical evidence, for within these populations geographical proximity was the only way of establishing cultural contact in the past. Furthermore, the material culture described here is not independent of ecological niche, especially because cultural distances are computed from information which includes subsistence.

A final observation is worth noting. Some researchers claim that a joint consideration of the linguistic and geographical divergencies at times indicates the presence of selective pressures, for example, linguistically distant but geographically close groups showing a great deal of genetic identity may be an indicator of selection as an evolutionary force³. In view of my analysis, such statements seem to be premature, for linguistic divergence can be the result of only recent historic developments of such tribal groups, whereas genetic differentiation by gene frequency changes is known to be a very slow process. Furthermore, in close proximity, exchange of a few mates between populations can never be ruled out. Theoretical predictions reveal that such migration need not be great to bring two neighbouring populations genetically close together¹⁶.

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Undulatory swimming with and without waves of contraction

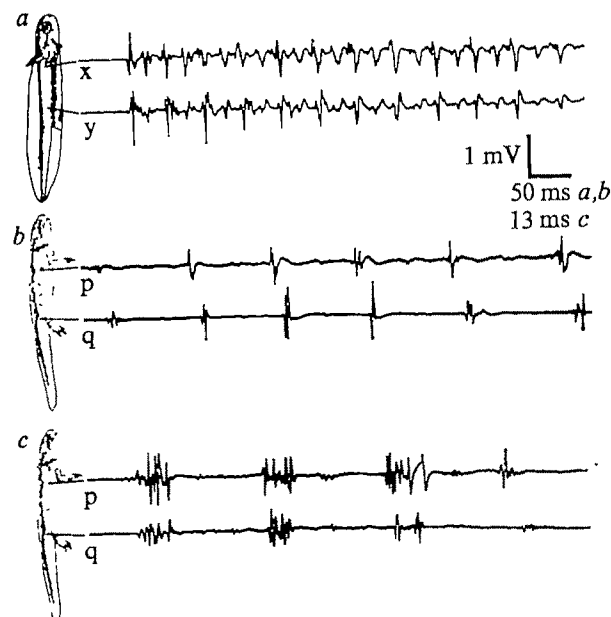
IT HAS been accepted generally that the waves of bending observed in swimming vertebrates are produced by contraction on the concave side of each bend of the body at any point within the cycle of movement¹⁻³. "Waves of contraction" are imagined to pass down the serial myotomes with the velocity of the propagated bends. Another concept, designed primarily to explain snake locomotion⁴, differs by a 90° shift in the timing of contraction on the bending wave, accounting for propulsion but resulting in illogical analysis of fish movement⁵. The former interpretation gives no explanation of the source of locomotory thrust, assuming falsely that the muscular activity required for a given body position is similar whether the position is held or passed through in locomotion. The dominant responsibility of locomotory activity is the propagation rather than the formation of bends, but how is propagation achieved against external resistance? Relevant electromyography of spinal

dogfish^{6,7} has not been related to this mechanical problem. I present here a preliminary report of electromyographic studies of developmental stages of the palmate newt, *Triturus helveticus* (Razoumowsky), and adult tench, *Tinca tinca* L., which show that undulatory propulsion in some animals does not involve waves of contraction and that the "waves" recorded in others function in reducing lateral oscillation anteriorly and adapting tail flexibility to different swimming speeds.

From the time when swimming by waves of bending is first possible in the newt embryo until the time of normal hatching there is no longitudinal delay in muscle activity along the body during the tailbeat. The electromyograms suggest that only the anterior myotomes are used (not those in the tail nor, at first, those in the lower trunk) and these contract together on either side (Fig. 1a). Recent ultrastructural studies have suggested that there is some electrical couplings between myotomal muscle fibres in amphibian embryos which persists for a short time after the development of swimming⁸⁻¹¹. Such coupling may be involved in the synchronisation of contraction along the trunk. The boundary between myotomes which seem to be active and those which seem to be inactive in swimming moves slowly back to the junction of trunk and tail by the end of embryonic life. But the inactivity of the more posterior myotomes survives the development of the capability for involvement in struggling movements, suggesting that it is a functional rather than merely a developmental feature.

The mechanics of this form of locomotion are easy to understand in outline from the simple oscillation of a flexible plate under water. Bending moments can be genera-

Fig. 1 Electromyograms of swimming activity in *T. helveticus*, recorded with 25- μ m diameter wire electrodes (tip position indicated on the left). a, (Monopolar) from a stage-37 embryo, 7.4 mm long; b and c, (bipolar) from a stage-55c larva, 29 mm long (see ref. 8 for developmental stages). In the thinner embryo the contralateral muscle activity is recorded at about half the amplitude of that from the side in which the electrodes are implanted. In the larva the contralateral activity is of much lower amplitude in the recordings. In (a) each of the large amplitude, spike-like events corresponds to a beat of the tail to the right, each of the lower amplitude intervening events to a beat to the left. There seems to be no delay in the occurrence of muscle activity down the length of the body comparable with that in (b) where there is a clear delay in the arrival of activity at electrode q after its cessation at electrode p. c, Violent swimming activity in the same animal, with a return to simultaneity except in the last, low amplitude beat. (The vertical gain on the trace from electrode p is five times that on the other traces which correspond to the scale-bar.)



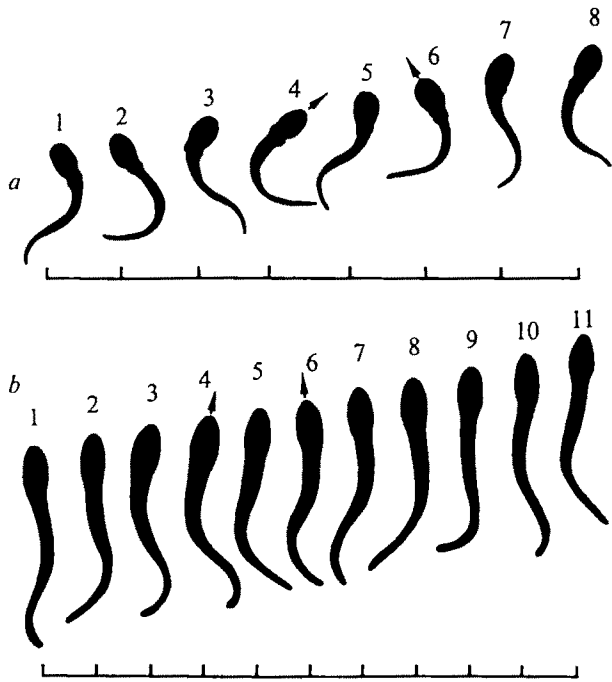


Fig. 2 Two series of tracings from ciné film of the swimming of *T. helveticus*: *a*, from a stage-37 embryo; *b*, from a stage-55c larva. Both sequences were filmed at 64 frames per s and the frame numbers for each sequence are given. Separation of each position from the baseline represents real movement in space. Each position has been displaced to the right from the preceding one by a fixed distance perpendicular to the baseline (shown by the scale divisions). The reduction of the oscillation of the head axis in (*b*) is indicated by the arrows showing the extreme positions at frames 4 and 6. The limbs have not been drawn in (*b*). The lengths of the animals are given in Fig. 1.

ted anteriorly and conducted along the tail by passive elastic tension. The form of the propagated waves will then depend on the animal's structural characteristics—particularly its flexibility and lateral hydrodynamic resistance—and their longitudinal distribution. The presence of a fin around the tail facilitates the development of both high flexibility and high lateral resistance in that region. These characteristics, combined with muscular passivity, can be seen to be responsible for the whip-like behaviour of the oscillated tail. In this early form of swimming the longitudinal axis of the head may swing through as much as 120° in the horizontal plane within the swimming cycle. During embryonic life the rotation of the head axis is reduced slightly (Fig. 2*a*) but is still measured in tens of degrees.

Early in larval life the myotomal contractions begin to show longitudinal delay, the propagation of some kind of "wave" (Fig. 1*b*). The characteristics of this wave are widely variable, as is the swimming behaviour of the larva, and in the most violent swimming the wave phenomenon is replaced by simultaneity of contraction along the myotomes (Fig. 1*c*). Associated with the wave of contraction is the reduction of the oscillation of the axis of the head to only a few degrees (Figs 2*b* and 4). In the newt larva, violent, "longitudinally simultaneous" swimming is accompanied by a return to wide lateral oscillation of the head.

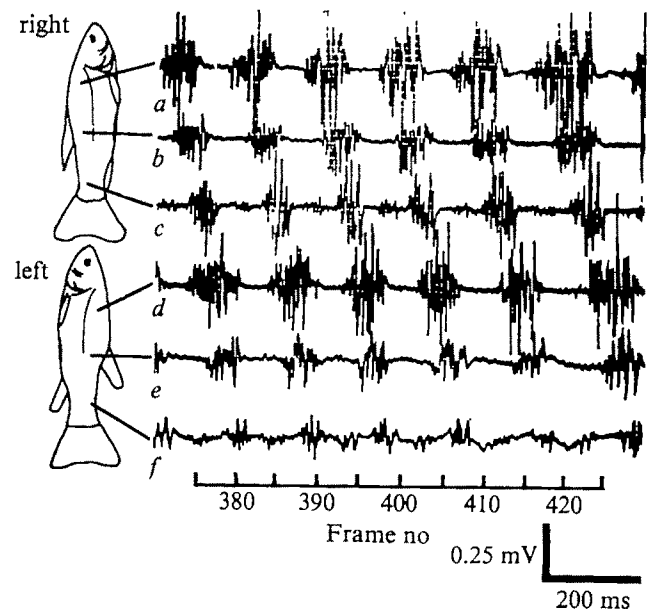
The wave phenomenon is more easily studied in a larger animal. The electromyogram of the tench in Fig. 3 shows that the delay in the initiation of muscle activity down the body is about twice that of the cessation. This means that the 'burst' of activity becomes shorter, moving caudally along the animal. There is also a period within each half-cycle of movement in which muscle activity is recorded simultaneously over the major part of the myotomal column. If the electromyogram is compared with the movements of the

animal (Fig. 4), periods of simultaneous lateral activity correspond with what may be called the S position of the body, the point at which conventional theory would least predict it. The contralateral overlap of activity occurs in those parts of the cycle in which the fish is approaching the C shape which is fully realised in the swimming of the newt embryo (Fig. 2*a*), but not quite achieved in the swimming of the tench (or the gentle swimming of the larval newt—Fig. 2*b*).

So although waves of contraction are not necessary merely for the propagation of waves of bending, they are recorded in the adult swimmer and they require an alternative explanation. The early initiation of contraction on one side of the anterior trunk, while the tail is still moving towards the other side, prevents the full lateral deflection of the head (Fig. 4). A similar effect is achieved by the newt larva in Fig. 1*b* with early relaxation anteriorly without contralateral contraction. This reduction of anterior movement may have a hydrodynamic significance¹², and certainly will have a behavioural significance in stabilising the cephalic sensory reception during locomotion, particularly in animals which hunt active prey.

In combination with this anterior function of the wave is the more complex concern with providing for various frequencies of tailbeat. The passive tail of the embryo would only function properly over a narrow range of frequencies suited to its mechanical characteristics, and this seems to be all that it is required to do. The embryo swims only at relatively high frequencies (usually 15–25 Hz). The activity of the tail muscle of the more developed animal has to alter the mechanical properties of the tail for wave conduction at different frequencies, for different frequencies entail different lateral velocities and accelerations, which in turn require different bending moments to be generated in the tail if the effective waveform is to be maintained. To do this economically, the mechanical properties of the tail must be suited for passive conduction of waves at the lowest frequencies required (perhaps 1 or 2 Hz) and then be 'stiffened'

Fig. 3 A 1-s portion of electromyogram from a free swimming tench during moderately fast cruising, recorded with six bipolar wire electrodes. The four forward electrodes were close under the skin, those in the tail peduncle (*c* and *f*) were deeper (so that they show something of the contralateral activity). The electrode tip positions are indicated on the left. The frame numbers of the horizontal axis refer to the ciné film which is partly traced out in Fig. 4. There is a delay of about 1.0 s m^{-1} in the initiation of muscle activity down the body, which is about twice the delay in its cessation (about 0.5 s m^{-1}). The overall length of the fish was 16.5 cm.



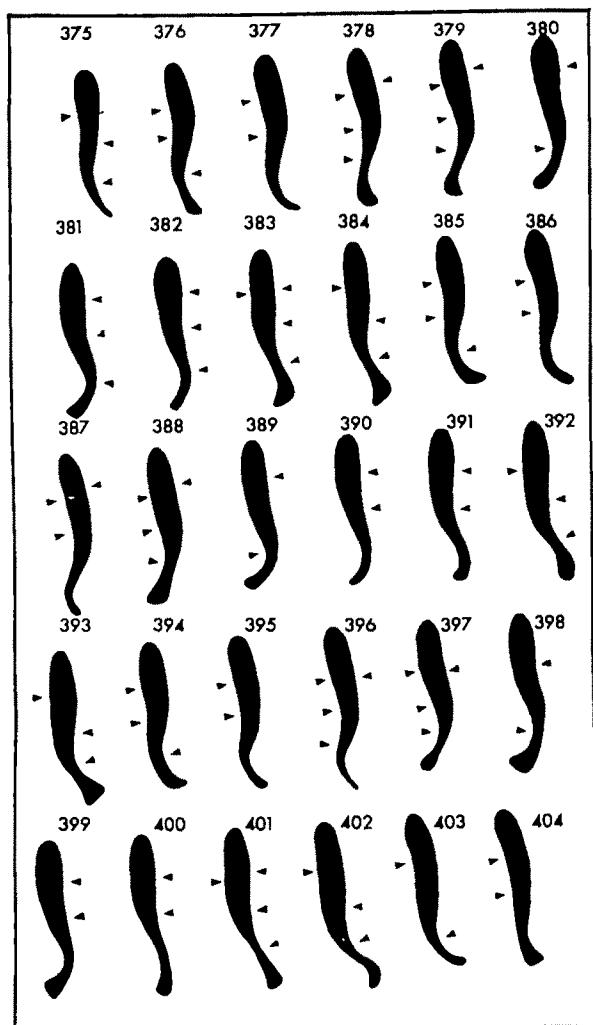


Fig. 4 A series of tracings from ciné film of the swimming of the adult tench electromyographically recorded in Fig. 3. The frame numbers above each position correspond to the scale given in Fig. 3. The arrowheads indicate the approximate sites of electrodes which were showing electrical activity in the muscle at the time of exposure of the film. (The form of the fish is at times slightly distorted by disturbance of the water surface.) The S position of the swimming cycle is characterised by activity only on one side or all down one side. The occurrence of contraction at the anterior of one side and the posterior of the other is associated with the approach of the tip of the caudal fin to its extreme lateral displacement. This condition is often preceded by activity recorded anteriorly appearing on both sides simultaneously. Camera speed was approximately 64 frames s^{-1} .

by muscular contraction during faster swimming movements. In this way the level of muscle activity is directly proportional to speed, rather than inversely, as would be the case if the tail were mechanically suited to the highest speed and actively flexed for lower frequencies. When these adaptations are incorporated, the wave of bending is produced partly passively, partly actively, and the role of the wave of contraction becomes complicated by involvement in modification of the propagation of the bend for the subtle control of movement.

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Effect of caffeine on coffee drinking

CAFFEINE is one of the world's most widely-used drugs and coffee drinking is a major form of its use¹. The levels of caffeine intake in coffee drinking have significant, predominantly stimulatory, physiological and behavioural effects². Surprisingly, the role of caffeine in the self-administration of coffee has not yet been studied. This research indicates that coffee drinkers drink more low-caffeine coffee than non-decaffeinated coffee.

In study 1, Instant Maxwell House Coffee (3.44% caffeine) and Instant Sanka Brand 97% Caffeine Free Coffee (0.14% caffeine) were mixed to produce doses of 100, 50, and 25 mg per cup of coffee. The coffees were packaged in individual-serving foil pouches of 2.94 gm. To avoid severe withdrawal symptoms (headache, fatigue or irritability)^{3,4} and to provide the possibility of maintaining normal caffeine consumption, smaller caffeine doses were avoided. (About 33 cups of decaffeinated coffee are needed to give 100 mg caffeine—the non-decaffeinated dose.)

Subjects were told that they would be receiving different types of coffee without additives. The study took 3 d for each subject—a day on each caffeine dose. The order of presentation was balanced. Daily, subjects received a generous, counted supply of coffee packets and a short questionnaire to assess how they were feeling and how the coffee tasted (good–bad, weak–strong, bitter–sweet, on four-point bipolar scales). Unused and used packets were to be returned. A count of the packets gave a check on reports of coffee intake. Consumption of other coffee, any tea, cola drinks, cocoa, chocolate and certain headache preparations was forbidden: all contain caffeine.

The volunteer subjects (seven females, five males) had an average age of 41 (range: 27–66) and by pre-experimental reports drank 4.5 cups of coffee per day (range: 3–6.5).

Table 1 shows a tendency for coffee intake to increase as caffeine content decreases. Subjects did not 'taste' the caffeine manipulation ($P > 0.5$). Changes in an index of how well the subjects were feeling (by self-report) correlated with changes in coffee consumption. The increased intake

Table 1 Average coffee intake (cups per person) as a function of the caffeine content of the coffee in three studies (\pm s.d.)

Study	Caffeine content (mg)		
	100	50	25
1	4.1*	5.0*	4.5
	± 1.2	± 1.7	± 1.1
2	1.2††	2.0†	2.1†
	± 0.43	± 0.06	± 0.06
3	2.6§	3.0§	3.1
	± 0.09	± 0.19	± 0.15

Means with same superscripts are different by two-tailed *t* tests, $P < 0.05$. In study 1, *t* tests for correlated samples were used; mean combined 50- and 25-mg intake was greater than 100-mg intake, $P < 0.05$; the unexpected quadratic trend, $F(1,22) = 6.10$, $P < 0.05$, may arise from debility from headaches in three subjects on 25-mg caffeine; if they are excluded, the trend disappears and 25- and 50-mg coffee intake becomes almost identical. In studies 2 and 3, *t* tests for independent samples are used for a rough estimate of statistical significance⁵.

of 50-mg relative to 100-mg coffee was associated with increased well-being ($r(10) = +0.70$, $P < 0.02$), the decreased intake of 25-mg relative to 50-mg coffee was associated with decreased well-being ($r(10) = +0.67$, $P < 0.05$).

Studies 2 and 3 treated an institutional coffee pot as the 'subject' in a single subject experiment. If the drinkers comprise constant groups, then the rate of emptying the pots—as a function of caffeine doses—is a valid measure of caffeine's effect on coffee intake.

Study 2 lasted from 0800 to 1100—the 'breakfast pot'—on Wednesdays and Thursdays for 4 weeks on a 40-cup coffee pot located in the dining room of a university hall of residence. There were about 12 regular coffee drinkers (six males, six females—all undergraduates). Roast and ground Maxwell House Coffee and Sanka Brand 97% Caffeine Free Coffee were mixed to yield 100-, 50-, and 25-mg caffeine doses per cup of coffee (week 1=100, week 2=50, week 3=25, and week 4=100 mg). The coffee left in the pot was measured with a ruler. Table 1 shows the estimated average coffee intake per person (each person weighted equally).

Study 3 used the 70-cup coffee maker used by ~ 25 secondary school teachers (~ 66% are female). The study lasted 3 weeks (week 1=100, week 2=25, and week 3=50 mg), from 0800 to 1200 on Tuesdays, Wednesdays, and Thursdays. Subjects were not asked to change their usual drinking habits: many had coffee before coming to work. Table 1 shows the results (adjusted for teacher absenteeism).

These studies were combined by transforming the results to standardised (or z) scores, see Fig. 1. Caffeine is shown to be a determinant of the frequency of coffee consumption. Though the regulation of caffeine intake is far from perfect (total caffeine intake decreases), it is impressive, on considering a few factors. Habits of intake may be slow to

change. Coffee drinking is also water drinking: water regulatory systems could prevent increases in fluid intake. Perhaps non-decaffeinated coffee provides surpluses of caffeine, such that 50-mg coffee ('half-caffeine' coffee) with only small increases in consumption is able to satisfy actual caffeine needs⁶. Too small doses of stimulants have been known to not sustain self-administration⁷: this may explain the failure of 25-mg coffee to be consumed more than 50-mg coffee. A half-caffeine mixture might be recommended to those who are trying to cut down on caffeine intake, but who still desire some of the benefits of caffeine.

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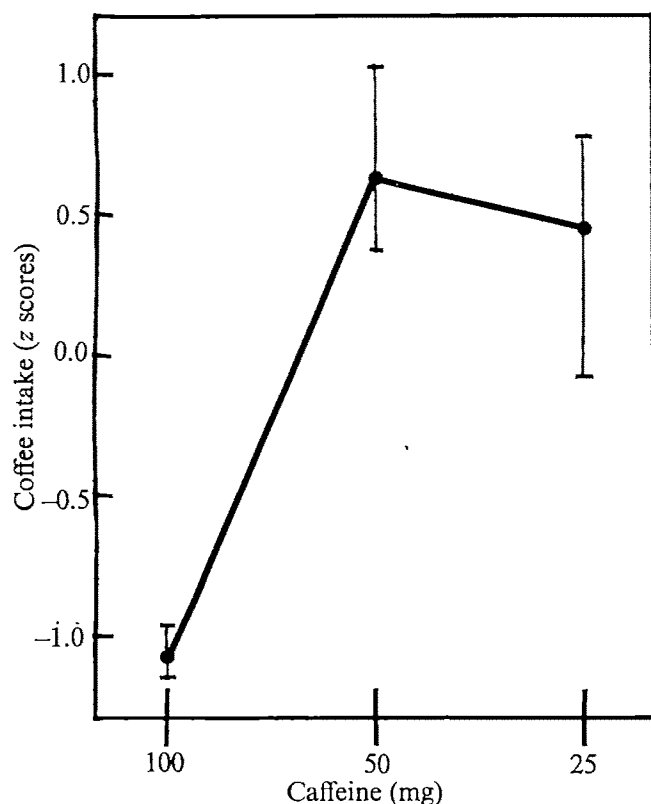
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Fig. 1 Standardised coffee intake scores (mean = 0, s.d. = 1) on average number of cups of coffee drunk per person as a function of caffeine content of coffee (log scale). Each study was standardised separately, and the range is indicated. The caffeine manipulation influenced coffee intake ($F(2,4) = 16.01$, $P < 0.02$). 50-mg coffee intake was greater than 100-mg coffee intake, $P < 0.02$; 25-mg intake was greater than 100-mg intake, $P < 0.05$; 50- and 25-mg coffee intake did not differ.



Vaccination against bovine tropical theileriasis (*Theileria annulata*)

SINCE the reports of Tsur¹ and Brocklesby and Hawking² on the multiplication of *Theileria annulata* schizonts in spleen explant cultures in plasma clot, the schizonts have been grown successfully for months in tissue culture monolayers^{3–5} and suspension cultures⁶. Successive cultivation of schizonts in tissue culture has been shown to bring about attenuation of the parasite^{7–10}, and suspensions of attenuated schizonts have been used as vaccine to immunise cattle against *T. annulata* in Israel⁴ and Iran¹¹. Reports^{4,11} indicate that the protective effect of the vaccine has been tested against challenge with schizonts but not against the natural tick-induced infection. As there may be some difference in the antigens of schizont and sporozoite (the injected stage of the parasite in the tick), we have evaluated the immunity produced by the vaccine against the infection caused by tick infestation.

Schizont-infected lymphoid cells were obtained by biopsy of a lymph gland of a calf infested with the tick *Hyalomma dromedarii* and infected with virulent Hissar strain¹² *T. annulata*. The cells were cultured in Eagle's MEM (Hanks' base), supplemented with 10% calf serum. Subcultures were made every second or third day and vaccine was prepared from the fifteenth passage.

Crossbred male calves reared in tick-proof rooms were vaccinated when two months old. Fifteen calves were injected subcutaneously at the base of the ear near the parotid lymph node with 1×10^6 schizonts of the fifteenth subpassage. After 35 d, they were challenged with the homologous strain of *T. annulata* by infestation with 10 infective ticks (*H. dromedarii*). The ticks were released on the ear, which was enclosed in a cloth bag. Eight similarly raised susceptible control calves were also infected.

The rectal temperature of the calves was recorded daily. Giemsa-stained smears of blood were examined daily, and of the biopsy material from the regional parotid lymph node

Table 1 Course of infection induced by inoculation of infected lymphoid cells

Calf no.	First onset of fever (d)	Duration of fever (d)	Maximum temperature (°C)	Commencement of swelling of the regional parotid lymph node (d)	% Schizont-infected cells in the lymph node biopsy material	First appearance of intraerythrocytic piroplasms (d)	Maximum parasitaemia
6	17	2	39.5	No swelling	Nil	—	Nil
7	No fever	—	—	No swelling	Nil	—	Nil
8	No fever	—	—	No swelling	Nil	—	Nil
9	No fever	—	—	No swelling	Nil	—	Nil
10	No fever	—	—	No swelling	Nil	—	Nil
200	No fever	—	—	No swelling	Nil	29	<1
191	13	5	40.4	No swelling	Nil	24	<1
192	15	1	39.4	No swelling	Nil	29	<1
193	15	4	40.0	No swelling	Nil	17	<1
197	16	4	40.0	No swelling	Nil	—	—
194	13	5	39.7	15	Nil	17	<1
195	15	4	40.4	15	<1	16	<1
196	13	6	40.4	15	Nil	20	<1
198	16	1	40.0	15	Nil	20	<1
199	15	2	40.1	15	Nil	—	—

Table 2 Course of *T. annulata* infection induced by infestation of unvaccinated control calves

Calf No.	First onset of fever (d)	Duration of fever (d)	Maximum temperature (°C)	Commencement of swelling of the regional parotid lymph node (d)	% Schizont-infected cells in lymph node biopsy material	First appearance of intraerythrocytic piroplasms (d)	Maximum parasitaemia (%)	Results
11	10	9	41.1	—	14	12	15	Survived
12	11	10	40.9	—	8	11	20	Died on day 25
13	11	10	41.0	—	12	12	25	Died on day 22
14	9	11	41.0	—	12	10	12	Survived
232	6	Until death	41.3	8	2	13	60	Died on day 15
234	9	Until death	41.1	8	25	13	80	Died on day 15
235	9	Until death	41.1	7	10	13	80	Died on day 15
237	9	Until death	41.1	7	10	13	80	Died on day 16

on alternate days beginning a week after the vaccination/challenge. Haemoglobin (Hb) content and packed cell volume (PCV) were determined twice a week.

The reactions to vaccination are summarised in Table 1. Ten of fifteen calves developed fever which commenced between days 13 and 16 and lasted for 1–6 d. The maximum temperature was 40.4 °C. Transient swelling of a regional parotid lymph node was observed in five calves. Parasitised lymphocytes (less than 1%) in the regional lymph gland appeared only in one calf (No. 195) on day 17. Intraerythrocytic piroplasms were observed in 8 of 15 calves between days 17 and 29 for the first time, and later at irregular intervals. Parasitaemia always remained less than 1%. Hb content and PCV of all calves remained normal, and all the calves survived.

When challenged, immunised calves showed no clinical or parasitological reaction, except for one calf (No. 199) which showed less than 1% parasitaemia on day 16. Hb content and PCV remained normal and the blood and regional lymph gland remained free of the parasites. But all eight calves of the control group suffered from the acute type of the disease and six of them died between days 15 and 25 (Table 2). The disease was characterised by high fever which started in 6–11 d, and lasted for 9 d or until death. The intraerythrocytic form of the parasite appeared in 10–13 d. Peak parasitaemias varied from 12 to 80%. The regional lymph gland was swollen in four of eight calves, although infected lymphoid cells (2–25%) were seen in the gland of all the calves.

We conclude therefore that all 15 vaccinated calves became fully immune to the severe tick challenge which killed six of eight unimmunised calves.

The vaccine produced no reaction in five calves and very mild clinical and/or parasitological reaction in the remaining 10, as also observed by Pipano and Israel⁹ with their

10th–15th passage material which at the 50th–120th passage produced no reaction. We are continuing to subculture the parasite with a view to further attenuation.

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Non-adaptive rejection of small tumour inocula as a model of immune surveillance

THOMAS's thesis¹ that it is a "universal requirement of multicellular organisms to preserve uniformity of cell type", led him to suggest that the phenomenon of homograft rejection represented the primary mechanism for natural defence against neoplasia. Burnet² viewed this surveillance of lethal oncogenic mutations as a function of the thymus-dependent adaptive immune system. Prehn^{3,4} and others^{5–7} have criticised the Burnet model of immune surveillance because it is unable to account for several important observations: (1) the adaptive

Table 1 Mice rejecting small syngeneic inocula in a primary challenge fail to exhibit memory on secondary challenge

Experiment	Tumour	Route of administration§	Inoculum size	%Cloning efficiency¶ ±s.e.m.	Turnour incidence on primary challenge		Large inoculum second challenge**		Small inoculum second challenge††	
					Number	%	Latency† (d±s.e.m.)	Survival (d±s.e.m.)	Number	%
1.	L5178Y*	Subcutaneous	5 × 10 ¹	47 ± 5	4/15	26	—	—	—	—
2.	L5178Y*	Subcutaneous	5 × 10 ¹	25 ± 7	8/40	20	12.5 ± 2.0	35.4 ± 1.0	—	—
		Intraperitoneal	5 × 10 ¹	25 ± 7	15/15	100	—	—	—	—
		Control	0	—	0/15	0	13.5 ± 2.5	36.4 ± 2.2	—	—
3.	P-815-X2*	Subcutaneous	5 × 10 ¹	44 ± 2	11/40	27.5	8.0 ± 1.0	33.8 ± 1.3	—	—
		Intraperitoneal	5 × 10 ¹	44 ± 2	15/15	100	—	—	—	—
		Control	0	—	0/15	0	7.9 ± 1.0	32.1 ± 2.3	—	—
4.	L5178Y†	Subcutaneous	10 ¹	—	1/8	12.5	—	33 ± 2	—	—
		Subcutaneous	5 × 10 ¹	—	2/8	25	—	40 ± 3	—	—
		Subcutaneous	10 ²	—	3/8	37.5	—	33 ± 5	—	—
		Control	0	—	0/5	0	—	45 ± 2	—	—
		Intraperitoneal	10 ¹	—	13/15	87	—	—	—	—
5.	P-815-X2†	Subcutaneous	10 ¹	—	4/8	50	—	38 ± 6	—	—
		Subcutaneous	5 × 10 ¹	—	2/8	25	—	39 ± 3	—	—
		Subcutaneous	10 ²	—	8/8	100	—	—	—	—
		Control	0	—	0/5	0	—	41 ± 5	—	—
		Intraperitoneal	10 ¹	—	8/8	100	—	—	—	—
6.	SL2†	Subcutaneous	10 ¹	—	3/8	37.5	—	37 ± 6	—	—
		Subcutaneous	5 × 10 ¹	—	6/8	75	—	32 ± 5	—	—
		Subcutaneous	10 ²	—	4/8	50	—	30 ± 7	—	—
		Control	0	—	0/5	0	—	35 ± 3	—	—
7.	1509a*	Subcutaneous	10 ²	—	3/20	15	—	—	3/14	21
		Subcutaneous	10 ³	—	5/20	25	—	—	2/10	20
		Subcutaneous	10 ⁴	—	13/20	65	—	—	3/6	50
		Subcutaneous	10 ⁵	—	20/20	100	—	—	—	—
		Intraperitoneal	10 ²	—	5/5	100	—	—	—	—
		Intraperitoneal	10 ³	—	5/5	100	—	—	—	—
		Intravenous	10 ³	—	0/10	0	—	—	—	—

*Tumours obtained from *in vitro* cultures for both primary and secondary challenge.

†Tumours obtained from ascitic passage for both primary and secondary challenge.

‡Appearance of palpable tumour after subcutaneous inoculation.

§All inoculations were 0.1 ml of washed cells. Subcutaneous inoculations were made in mid-low back after removing the fur.

¶After thorough washing, the tumour was resuspended in Fischer's medium containing 15% foetal calf serum. Aliquots of 2.0 ml containing 5 × 10¹ cells were added to 3 ml of 2% Noble special agar at 44 °C. After thorough mixing, the suspension was placed in a 37 °C incubator at 5% CO₂ in a humid atmosphere. Visible clones were counted after 2 weeks in culture. Cloning efficiency was calculated from a minimum of 10 replicates.

**An aliquot of 10⁵ tumour cells in 0.1 ml Fischer's medium was inoculated subcutaneously in the same site as the primary challenge.

††Tumour frequency was assessed 25 d after subcutaneous challenge with the same number of tumour cells as the primary inoculum.

immune system, with only rare exceptions, is unable to eliminate incipient or induced tumours rather than functioning as a surveillance mechanism^{4,5-8}; (2) the immune response to tumours is as likely to result in immunosuppression and enhancement of tumour growth as the destruction of those tumours^{6,9}; and (3) the incidence of spontaneous tumours in athymic mice is insignificant⁸. To explain these data one must either assume that surveillance does not exist, or one must postulate that surveillance exists in a form other than the thymus-dependent adaptive immune response. We have attempted to examine the hypothesis that surveillance exists and is a non-thymus-dependent phenomenon by an experimental model in which the fate of a small tumour inoculum, simulating an oncogenic mutation, is examined after manipulation of the host's immune response.

Several carcinogen-induced tumour lines, the 1509a fibrosarcoma, P-815-X2 mastocytoma and the SL2 lymphoma, as well as the spontaneous lymphoma L5178Y, when implanted subcutaneously in small numbers fail to produce death or a palpable tumour mass in a large proportion of syngeneic recipients (Table 1). To identify the number of injected cells capable of forming a tumour mass, medium containing 50 cells of L5178Y or P-815-X2 was placed in a soft agar gel which allows the formation of single cell clones¹⁰. Cloning efficiencies of up to 46% per inoculum were demonstrated after 3 week of culture (Table 1). The 1509a tumour cells were also shown, in repeated experiments, to grow well *in vitro*. Inocula of 10², 10³ and 10⁴ cells produced 5 × 10⁴, 10⁶ and 5 × 10⁶ tumour cells respectively, in 14 d. The capacity of the small tumour inocula to grow *in vivo* was demonstrated after intraperitoneal implantation of the 1509a, L5178Y and P-815-X2 (Table 1). In spite of the ability of 10² 1509a to grow intraperitoneally, intravenous

as well as subcutaneous challenge with that number of cells produced very few tumours (Table 1).

The rejection of the small subcutaneous inocula, however, could be prevented by the simultaneous intravenous injection of papain digest or 3 M KCl extracts of syngeneic tumour membrane (Table 2). Tumour frequency was 2.5–3.5 times higher than in untreated controls or controls receiving allogeneic membrane at the optimal dose of membrane extract.

The existence of memory was assessed in mice surviving the initial challenge of a small tumour inoculum by rechallenging with a larger tumour dose which was observed in preliminary experiments to result in 100% mortality. The latency and survival rate of mice which had previously rejected the L5178Y and P-815-X2 inocula was no different from that of normal control mice (Table 1). No difference was observed in the survival rates when mice were inoculated with tumour obtained from either *in vivo* peritoneal passage or *in vitro* tissue culture (Table 1). Similarly, mice which had rejected the 1509a in the primary challenge with the small inocula did not reduce either the growth rate or the mortality rate of the secondary challenge. It was also found that mice which had rejected 10² 1509a cells had the same tumour incidence as untreated controls when rechallenged with the same small inoculum (Table 1).

We examined the effects of suppression of the immune response on the incidence of tumours. A/J mice can be made resistant to 1509a by surgical removal of the primary tumour, and the subsequent simultaneous administration of suppressor T cells and 1509a to these immune mice results in enhanced tumour growth⁹. Suppressor T cells given with a small inoculum of 1509a in a primary challenge did not, however, result in any enhancement in the frequency of tumours or their growth rate. In addition, the simultaneous administration of suppressor

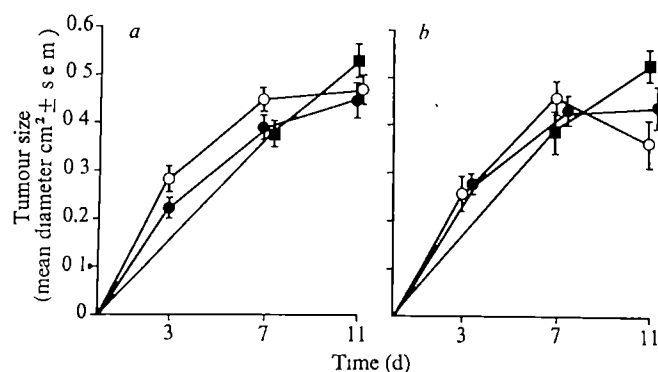


Fig. 1 Absence of memory after the rejection of a small 1509a tumour inoculum. *a*, Primary inoculation with 10^3 1509a subcutaneously was followed by a secondary challenge with 10^5 tumour cells 30 d later in the tumour-free mice. Suppressor T cells obtained from A/J thymus⁹ were given intravenously, simultaneously with the 10^5 inoculum in a second group of mice. Control mice did not receive a primary inoculation before 10^5 tumour challenge. The tumour size was measured with a Vernier caliper in two diameters at right angles. Observations were made on five mice in each experimental group. (●) 10^5 1509a secondary challenge after rejection in 10^3 primary challenge; (○) 10^5 secondary challenge after injection of a primary challenge with 10^3 1509a and 4×10^7 suppressor T cells; (■) 10^5 1509a primary challenge. *b*, Experimental protocol is identical to *a* except the primary inoculation is 10^5 1509a.

T cells and the small primary tumour inoculum did not enhance the growth rate of the secondary challenge of 1509a (Fig. 1).

We examined further the question of whether the rejection of small tumour inocula was a thymus-dependent adaptive immune phenomenon by subcutaneously implanting the 1509a and the L5178Y in their respective syngeneic hosts after adult thymectomy, lethal irradiation and bone marrow reconstitution. The frequency of tumours was slightly less in the immunodeficient mice than in the intact control mice, in spite of a much reduced survival rate when challenged with a large tumour dose (Table 3).

If the inoculation of a small number of tumour cells is an adequate model for an oncogenic mutation, the ability of immunodeficient or immunosuppressed mice to reject them suggests that surveillance is not a thymus-dependent adaptive immune phenomenon. This thesis is also supported by the additional evidence that the rejection is not associated with memory.

The ability of syngeneic tumour membrane extracts to

Table 2 Enhancement of tumour frequency after subcutaneous implantation of small tumour inocula by the intravenous injection of syngeneic tumour membrane preparations

Membrane preparation	Tumour* Amount† (µg)	Tumour inoculum	Tumour frequency‡
L5178Y	0.1	5×10^3 L5178Y	7/16 44
L5178Y	1.0	5×10^3 L5178Y	12/23 52
L5178Y	10.0	5×10^3 L5178Y	6/16 37
—	0	5×10^3 L5178Y	7/35 20
1509a	1.0	5×10^3 L5178Y	2/12 17
1509a	0.1	10^3 1509a	4/10 40
1509a	1.0	10^3 1509a	7/10 70
1509a	10.0	10^3 1509a	5/10 50
—	0	10^3 1509a	2/10 20
L5178Y	1.0	10^3 1509a	0/5 0

*Papain digest of L5178Y membrane was prepared by the method of Maramatsu *et al.*²¹. The membrane digest was centrifuged at 25,000 r.p.m. for 10 min and placed on a Sephadex G-200 column. A single protein peak of low molecular weight was observed. The 3 M KCl membrane extract of 1509a was prepared by the method of Forbes *et al.*²².

†1.4 = 0.7142 mg ml⁻¹ at E₂₈₀

‡Tumour frequency was assessed 25 d after the implantation of the subcutaneous tumour inoculum. The data represent a total of three experiments for the L5178Y and two experiments for 1509a.

Table 3 Frequency of tumours and mortality of adult thymectomised, lethally irradiated and bone marrow reconstituted mice (AT×BM) challenged with small and large tumour inocula

Experiment	Tumour	Inoculum size	Recipient†	Tumour‡ frequency No.	Survival %	Survival (d± s.e.m.)
1.	L5178Y*	5×10^3 5×10^3	Normal DBA/2 AT×BM DBA/2	3/15 0/15	20 0	> 40 —
2.	L5178Y	10^5 10^5	Normal DBA/2 AT×BM DBA/2	19/19 14/14	100 100	35±28 18±1
3.	1509a	10^3 10^3 10^3 10^3 10^5	Normal A/J AT×BM A/J Normal A/J AT×BM A/J Normal A/J	0/5 0/5 2/5 1/5 5/5	0 0 40 20 100	— — — — —

*Cloning efficiency of the L5178Y was $47 \pm 5\%$ per inoculum.

†AT×BM mice received 800 rad irradiation 4 weeks after adult thymectomy followed by 2.5×10^7 washed bone marrow cells intravenously. Mice were challenged with tumour 3–4 weeks later.

‡Tumour frequency was assessed at 30 d after inoculation of tumour.

§ $P < 0.001$

enhance tumour frequency suggests that the failure of tumour growth is due to a rejection mechanism with at least some specificity. It is also clear from our observations that one of the features characteristic of this mechanism is the rapidity of its response, acting well before the tumour has reached a critical size. Since tumour growth is very rapid and the time required to reach this critical size is theoretically very small, it suggests that the recognition mechanism is functionally optimal in the normal animal and capable of acting before an adaptive immune response can be generated. Such pre-existing specific immune mechanisms have been identified in two forms in mammals: naturally occurring antibodies^{11,12} and naturally occurring cytotoxic cells^{13–18}.

Natural antibodies with specificity for spontaneous or carcinogen-induced tumour, such as were used in the present study, have been found in normal mouse sera as well as sera from congenitally athymic (*nu/nu*) mice and are predominantly IgM^{14,16}. The role of IgM in anti-tumour immunity is not entirely clear, but it has been implicated in cell-mediated immunity to murine sarcoma virus¹⁷ and tumours induced by mammary tumour virus¹⁸.

Naturally occurring cytotoxic cells have been demonstrated with well defined specificities in *in vitro* assays of tumours immunity^{13–15} and are under genetic control of a strong H-2-linked factor in mice¹⁹. These cells have been partially characterised and bear neither B- nor T-cell membrane antigens^{13,14,20}. Keissling *et al.*²⁰ have also recently demonstrated a correlation between *in vitro* cytotoxicity by naturally occurring cytotoxic cells and their ability to produce *in vivo* neutralisation of an MSV lymphoma in a Winn assay. It is not clear at this time whether mice possess these effector cells in sufficient heterogeneity to account for the recognition of the diverse populations of tumours expected as a consequence of carcinogen-induced mutations, viral transformation, or tumour induction by other oncogenes. But the possibility that these effector cells are responsible for the rejection phenomenon we have observed must certainly be entertained.

We believe, therefore, that if immune surveillance of tumours exists, the possibility that it functions as a thymus-independent, non-adaptive phenomenon must be considered. Although the nature of this type of a surveillance mechanism is not immediately apparent, its identification may be of considerable importance to tumour biology and immunotherapy.

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X chromosome activity in oocytes of kangaroo pouch young

In female eutherian mammals, inactivation of one of the two X chromosomes occurs randomly from cell to cell in somatic tissues¹ but in oocytes (meiotic germ cells) X inactivation does not occur for at least three loci. Oocytes of adult XX mice possess twice as much activity as those of XO mice for the sex-linked enzyme, glucose-6-phosphate dehydrogenase (G6PD)², hypoxanthine guanine phosphoribosyl transferase (HGPRT)³, and phosphoglycerate kinase (PGK)⁴. Furthermore, oocytes of adult⁵ and foetal⁶ human females, heterozygous for G6PD allelic genes, show activity for both fast and slow electrophoretic forms of the enzyme. In female kangaroos (Marsupialia: Macropodinae) inactivation of the paternally derived X chromosome occurs in cells of various somatic tissues⁷ but the question of X chromosome activity in oocytes has not been resolved. We have now examined G6PD expression in ovaries of pouch young known to be heterozygous for the fast and slow forms of the enzyme, and show that only a single X chromosome—the maternally derived one—is active in ovarian cells.

Inheritance of G6PD is sex linked in kangaroos⁸. Crosses between wallaroos (*Macropus robustus robustus*: G6PD-F) and euros (*M.r. erubescens* G6PD-S) produce fertile female hybrids heterozygous for G6PD type. Electrophoresis was carried out on ovaries from six heterozygous pouch young aged between 53 and 60 d *post partum*. This age class has the greatest number of oocytes during ovarian development of *Macropus eugenii*⁹ and we assume the same is the case for *M. robustus*. Graphical reconstruction of cell and tissue distribution from serial sections of an ovary from a 53-d-old pouch young showed that primary oocytes accounted for about 70% of the total ovarian volume, a far higher proportion than at any stage of human ovarian development¹⁰.

Most of the oocytes were in zygotene or pachytene of prophase of meiosis I. Specific staining¹¹ of frozen sections of an ovary from another heterozygous pouch young aged 57 d showed that oocytes had a high level of G6PD activity. Although we have no quantitative data on this point, it seems unlikely that activity levels differ greatly from those in human oocytes at comparable stages of differentiation.

If both alleles are functional in oocytes heterozygous for G6PD and the enzyme molecule is a dimer, then electrophoretic bands should be present in the fast and slow positions together with a stronger intermediate or heteropolymer band. In the ovarian extracts examined, however, only a single band was found occupying the maternal position regardless of the direction of the cross, and there was never any trace of a band in the paternal position (Fig. 1). A brief reference to two of these heterozygotes was made earlier¹².

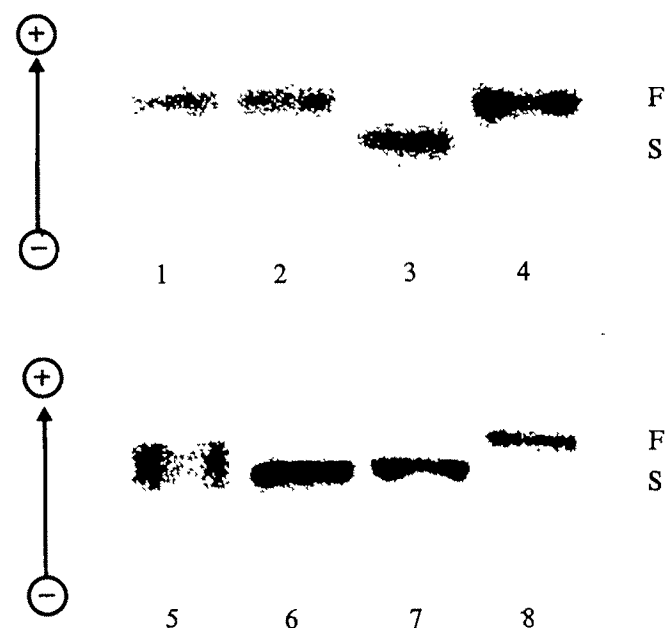


Fig. 1 G6PD electrophoretic phenotypes (F, fast; S, slow) from ovarian and other tissue homogenates of heterozygous pouch young. In the F/S heterozygote (age: 55 d) and the S/F heterozygote (56 d) the allele of maternal origin is referred to first. The S/S homozygote is used as a control. 1, F/S brain; 2, F/S ovary; 3, S/S ovary; 4, F/S uterus; 5, 1:1 mixture of F/S and S/F kidneys; 6, S/F ovary; 7, S/F oesophagus; 8, F/S oesophagus. Channels 1-4 and 5-8 represent separate gels. Samples were homogenised in an equal volume of lysing solution¹³ and applied on to CelloGel (Chemotron). Electrophoresis was carried out in 0.1 M lithium borate-0.0024 M EDTA buffer, pH=9.0, for 1.5 h with a voltage gradient of 14 V cm⁻¹. The gel stain was that of Rattazzi *et al.*¹¹.

In view of the preponderance of oocytes in the ovaries examined and their relatively high levels of G6PD activity, it seems reasonable to conclude that X inactivation of the allele of paternal origin occurs in these cells. At our level of detection it is most unlikely that gene product, forming bands in paternal and intermediate positions, could go undetected. We consider, therefore, that inactivation of the paternal G6PD allele is a feature not only of female kangaroo somatic cells but also of their early primary oocytes.

In human foetal ovaries, results of electrophoresis of G6PD heterozygotes were interpreted as evidence for a reactivation of the inactive X chromosome of germ line cells near the time of entry to meiosis¹³, rather than at the differentiation of primordial germ cells into oogonia as had previously been suggested¹⁴. Foetal ovaries with numerous

zygotene and pachytene oocytes gave a distinct heteropolymer band but in younger specimens with few leptotene oocytes, the heteropolymer band was weak or not detectable at all¹². Ovaries of heterozygous kangaroo pouch young, in which zygotene and pachytene oocytes were predominant, gave no trace of a heteropolymer band, indicating that if reactivation of the paternal allele occurs in kangaroo oocytes it occurs at a later stage of oogenesis. We are at present investigating adult heterozygote ovaries to establish whether reactivation has occurred by the dictyate oocyte stage.

That reactivation of the paternal allele for G6PD does occur at some time during oogenesis (or during embryogenesis) is supported by kangaroo pedigree data¹³. Progeny derived from G6PD heterozygotes may inherit either allele in an active form, suggesting that both X chromosomes are potentially active, at least at this locus. We assume, as have other workers, that activity states at the G6PD locus represent activity states at most if not all X-linked loci so that inactivation of the paternal allele for G6PD is indicative of inactivation of the paternal X chromosome. The patterns of transcription at other X-linked loci in kangaroo oocytes need to be elucidated to validate the supposition.

Kangaroo pachytene oocytes possess an XX bivalent which is isopycnotic with the autosomal bivalents. Air-dried preparations showed no differential condensation of either homologue or regions of homologues. There is thus no cytological evidence of a difference in genetic activity between the maternal and paternal X chromosomes of the kangaroo sex bivalent.

The mechanisms which control the maintenance of X inactivation early in oocyte differentiation and the onset of reactivation at some later stage are not known and are worthy of investigation in the more general context of regulation of activity states in chromosomes.

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Mutagenesis and transformation of normal cells by chemical carcinogens

TREATMENT of normal cells¹⁻⁶ or some established cell lines⁷ in culture with chemical carcinogens can result in cell transformation and malignancy. Cells which have an oriented

pattern of cell growth, a limited life span *in vitro* and are not tumorigenic *in vivo*, are then converted into cells with a hereditary random pattern of cell growth, an ability to grow continuously in culture and to form tumours *in vivo*. Studies with a variety of polycyclic hydrocarbons and other chemicals have shown a correlation between the frequency of colonies with a hereditary random pattern of cell growth (transformed colonies) and the degree of carcinogenicity in animals^{2,6,7}.

The analysis of cell transformation by benzo[a]pyrene, a prototype and most common carcinogenic polycyclic hydrocarbon, has indicated that transformation by this carcinogen is a one-hit event⁸. This one-hit response and the observation that carcinogens can bind to cellular DNA^{9,10} suggest that transformation may result from a single alteration in the genetic constitution of the treated cell. Carcinogens may, therefore, transform cells as a result of a somatic mutation. It has indeed been found that chemical carcinogens can produce somatic mutations at various genetic loci¹¹⁻¹⁵ and that there is a relationship between the degree of carcinogenicity and mutagenicity with different chemicals¹²⁻¹⁵. The relationship between carcinogenesis and mutagenesis in these studies was not, however, tested with the same cells, nor was mutagenesis by environmental carcinogens tested with normal diploid cells.

To elucidate the mechanism of carcinogenesis, it is important to determine the number of the same or different genes which may be involved in cell transformation. The frequency of cell transformation and mutagenesis of specific loci can both be tested *in vitro*. We have, therefore, now carried out experiments with normal diploid cells to determine whether the ratio between transformation and mutation of a specific locus is small, about 10, or large, more than 10³. We have determined mutation and transformation frequencies in primary cultures of normal golden hamster embryo cells treated with the carcinogenic hydrocarbon benzo[a]pyrene and its metabolite 7,8-trans-dihydrodiol¹⁶. The frequency of transformed colonies has been compared with the frequency of mutation for ouabain resistance, a mutation associated with the surface membrane Na⁺/K⁺ ATPase^{16,17}. This mutation was chosen because of its low spontaneous frequency and the ease with which it can be detected in normal diploid cells¹⁷. The cells were treated in the same way to test for the frequencies of transformation and ouabain-resistant mutants.

The results have shown that benzo[a]pyrene and its 7,8-dihydrodiol can induce both transformation and mutagenesis for ouabain resistance in normal diploid cells. The ratio between transformation and mutagenesis for ouabain resistance was about 20:1 with both hydrocarbons (Table 1). Other studies with normal hamster embryo cells treated with benzo[a]pyrene, have indicated a transformation-to-mutation ratio of about the same order of magnitude (J. C. Barrett and P. O. P. Ts'o, personal communication). Since ouabain resistance is presumably due to mutation at one locus¹⁷, it can be suggested that transformation as measured by the appearance of colonies with a random pattern of cell growth has a target equivalent to about 20 such genes. There may thus be this number of the same or different genes, the mutation of any one of which is the single hit required for transformation. Further studies with other genes can determine whether some genes show a lower or higher transformation-to-mutation ratio and whether this ratio changes with different types of carcinogens. The number of genes involved in transformation may be even smaller than about 20, if the genes for transformation are located at hot spots¹⁸ which have a higher mutation frequency. It is of interest that genes which determine the production of altered heavy chains of immunoglobulin in mouse myeloma cells also have a high mutation frequency^{19,20}, of the same order of magnitude as the frequency of cell transformation.

Table 1 Transformation and mutagenesis by benzo[a]pyrene and its 7,8-dihydrodiol

Hydrocarbon	Concentration ($\mu\text{g ml}^{-1}$)	Cloning efficiency (%)	No. of transformed colonies per no. of colonies counted	No. of transformed colonies per 10^6 colony-forming cells	No. of ouabain resistance colonies	A/B
				(A)	(B)	
None	—	12.5	0/6,736	—	0.04	—
Benzo[a]pyrene	1	1.2	16/3,753	4,300	213	20
7,8-dihydrodiol	0.1	2.6	25/5,309	4,700	193	24
	0.3	1.3	18/2,203	8,200	451	18
	1.0	0.9	20/3,183	6,300	419	15

Three- to five-day-old primary normal golden hamster embryo fibroblasts were seeded at 1.5×10^6 cells in 8 ml Eagle's medium with a four-fold concentration of amino acids and vitamins (H-21, Grand Island) with 10% foetal calf serum in 100 mm tissue culture (Falcon) Petri dishes. One day after seeding, the cells were treated for 3 d with benzo[a]pyrene or its 7,8-dihydrodiol metabolite dissolved in a final concentration of 0.5% acetone. The cells were then washed with medium, dissociated with trypsin solution and seeded to determine the frequency of transformed and ouabain-resistant colonies. To test for transformation the treated cells were seeded as described¹⁻³ and the number of colonies counted 10 d after seeding. To test for ouabain resistance¹⁴⁻¹⁷, the carcinogen-treated cells were seeded at 10^6 cells per 50 mm Petri dish. Two days later ouabain was added at a final concentration of 1 mM and the number of colonies counted after 18 d. In all experiments colonies were counted after staining with Giemsa. The data presented are the total results of four separate experiments per point. At the plateau concentration, there was up to about a fourfold variation in the assays for transformed and ouabain-resistant colonies. The heritability of the ouabain resistance was shown with five isolated ouabain-resistant colonies, all of which remained resistant to the drug after 10–12 d growth in the absence of ouabain.

We suggest that cell transformation is due to a mutation and that this mutation can occur in one out of a small number of the same or different genes. Cell transformation by chemicals resulting in a hereditary random pattern of cell growth is an initial step in the process that can result in the formation of tumours^{4,5}. In addition to the mutation resulting in transformation, it also seems that there can then be other genetic changes such as the selection of specific genes that control the ability of cells to form a tumour²¹⁻²³.

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Dipole potential measurements in asymmetric membranes

We report here detection of two kinds of asymmetry in planar lipid bilayers which may bear on the problem of asymmetry in biological membranes. One kind of asymmetry arises as a consequence of an asymmetric distribution in the bilayer of lipids with charged polar head groups, and the other from an asymmetric distribution of lipids with different but neutral polar

head groups. Several studies indicate that the lipids of the red cell membrane are asymmetrically distributed. In the erythrocyte membrane the outer half of the bilayer consists predominantly of neutral lipids, whereas the inner half contains all the negatively charged phospholipid phosphatidylserine¹⁻³. In nerve axons such lipid asymmetry has not been demonstrated directly but can be inferred from surface charge measurements. These measurements indicate that at least in the neighbourhood of the sodium channel, the negative charge density is greater in the outer monolayer of the membrane^{4,5}.

Recently it has been shown that lipid redistribution between the two halves of the bilayer is a very slow process⁶⁻⁸, thus indicating that membrane asymmetry can be a stable characteristic of biological membranes. A natural consequence of lipid asymmetry in membranes is a difference in surface potentials. In appropriate conditions this difference can have a major role in controlling ion transport^{9,10}. A surface potential may arise either from ionised groups or from oriented dipoles at the membrane surface or both, and there were indications that the dipole contribution is such that the membrane interior is >300 mV positive with respect to the external solutions¹⁰. Surface charge usually makes the membrane more negative with respect to the solutions. In previous work we showed that surface charge asymmetry in bilayer membranes can be detected and measured using the nonactin-K⁺ complex as a probe⁷. Here we report a similar measurement of the dipole potential difference between two lipids with different but neutral polar head groups. The potential measured in this case does not arise from surface charge, but from a difference in dipole moment per unit area in the two lipid monolayers which form the membrane.

The technique used to form asymmetric bilayers by apposition of the two separate monolayers has been previously described^{7,11}. In the present experiments one monolayer was formed from bacterial phosphatidylethanolamine (PE, Supelco, Inc.) and the other from 1,3 Diolein (GDO, Applied Science Lab., Inc., 99 per cent+) or bovine phosphatidylserine (PS, Supelco). The aqueous solutions in both compartments were equal and consisted of unbuffered (pH ~ 6) KCl solutions of indicated concentration. Nonactin (E.R. Squibb) was added to both compartments in concentrated ethanolic solution; the dilution was usually by a factor of 10^3 . Electrical measurements were made using Ag/AgCl electrodes in a four electrode system¹². Current-voltage curves were directly recorded on an X-Y recorder. The rate of change of voltage was 10 mV s⁻¹ or less to avoid hysteresis. All the experiments were made at $20 \pm 2^\circ\text{C}$.

Figure 1 shows a steady-state current-voltage curve of an asymmetric bilayer membrane in which one monolayer is made of GDO and the other of PE. The voltage is defined as positive

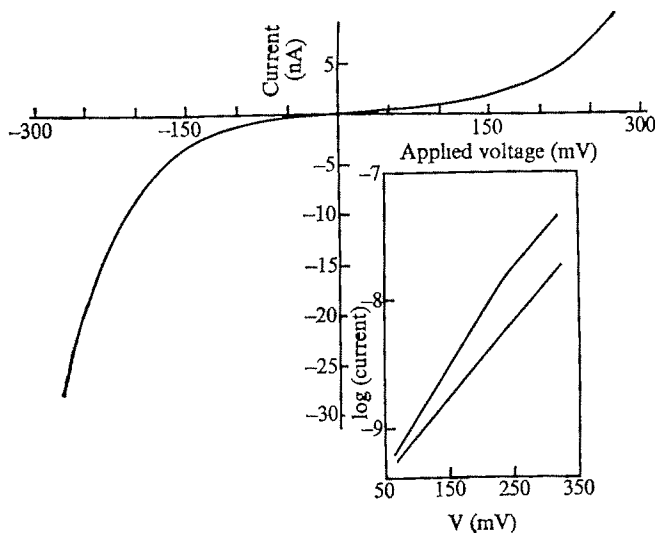


Fig. 1 Steady-state current-voltage curve for an asymmetric membrane made of GDO and PE. Nonactin concentration 2×10^{-8} M. 1 M KCl unbuffered ($\text{pH} \approx 6$). Temperature 21°C . Positive current is defined as flowing from the high dipole potential side (PE) to the low dipole potential side (GDO). The insert shows the data plotted on a logarithmic scale. Note the change in slope for current flowing from the GDO side to the PE side for negative potential in the upper curve. The lower curve is for positive potentials and current flowing from the PE to the GDO side.

when current flows from the PE side across the membrane to the GDO side. The rectification shown by the current-voltage curve is consistent with a more positive dipole potential on the PE side. This rectification arises as a consequence of the actual energy barrier to transport inside the membrane, but it is easier to understand with the trapezoidal approximation shown in Fig. 2. We have previously shown that for large applied voltages a trapezoidal approximation of the actual barrier profile is adequate to describe the steady state current-voltage curves in bacterial PE membranes¹³ as well as those found in asymmetric membranes made of bacterial PE and phosphatidylserine⁷. Figure 2a shows the trapezoidal approximation for the energy barrier profile expected for an asymmetric membrane with a difference in dipole potential, $\Delta\phi$, across it. Studies made in

both monolayers¹⁰ and symmetric bilayers^{10,14} indicate that $\phi\Delta$ between GDO and PE is ~ 100 mV, the interior of a PE membrane being more positive. As shown in Fig. 2b the rate limiting energy for the currents in the positive direction, ϕ_{PE} , is reduced by a positive voltage, nV , where n is the fractional distance to the corner. On the other hand, the rate-limiting energy for the current in the negative direction, $\phi_{\text{GDO}} + \Delta\phi$ in Fig. 2c, is reduced by a negative voltage, $(1-n)V$. Accordingly, the currents in the positive and negative potentials at large voltages must be proportional to $\exp(neV/kT)$ and to $\exp[e(1-n)V/kT]$ respectively, and we would expect a difference in the functional dependence of the current on voltage for different sign of applied voltage like the one shown in Fig. 1. Differences in dipole potential can be calculated with ~ 5 -mV accuracy from the differences in functional dependence of the current on voltages of different polarities. The insert of Fig. 1 shows the logarithm of the current against voltage. The upper curve in the insert represents current flowing from the GDO side to the PE side and the lower curve the reverse current. Both curves diverge until a voltage at which the upper curve changes its slope and becomes parallel to the lower curve. At this point in voltage the external applied potential has compensated exactly for the differences in dipole potential between the two lipids. The calculated difference in dipole potential is 107 mV in 0.02 M and 101 mV in 1 M KCl. It is important to point out that no rectification in the current-voltage curve is expected from a triangular barrier, a shape frequently assumed in the study of carrier mediated transport.

We previously reported⁷ asymmetry in membranes formed with one monolayer of PE and the other of PS. Figure 3 shows the change with ionic strength in the surface potential difference of both PE-PS membranes and PE-GDO membranes. The PE-PS membranes show an asymmetry which depends on ionic strength as predicted on the Gouy-Chapman theory. The asymmetry of PE-GDO membranes is, however, independent of ionic strength. This observation is in agreement with the fact that PE and GDO are neutral at the pH used ($\text{pH} \sim 6$). This lack of change in surface potential with a change in ionic strength is an important characteristic of surface potentials arising from dipoles rather than surface charge.

Differences in dipole potential between different lipids have been inferred by measuring and comparing the specific conductance of symmetrical membranes in the presence of non-actin¹⁸ or lipophilic ions⁹. In the absence of space charge and

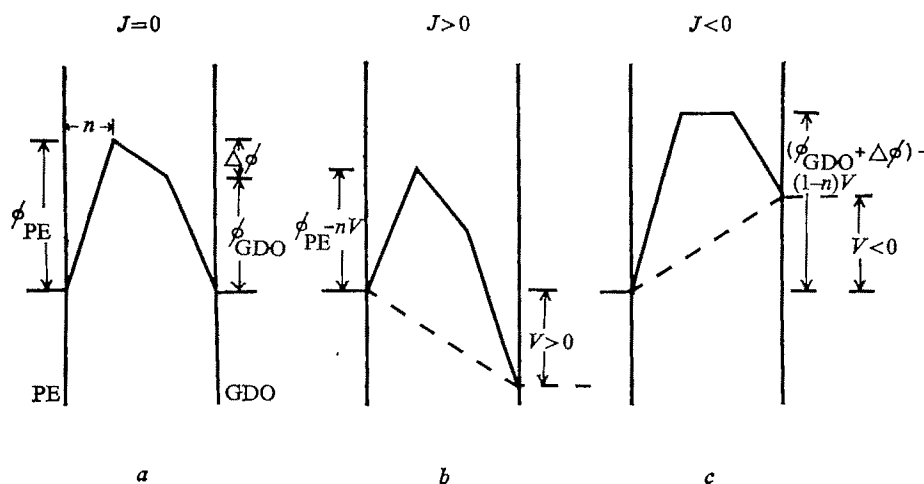


Fig. 2 Schematic representation of the energy barrier of an asymmetric membrane made of GDO-PE. ϕ_{PE} is the barrier height 'seen' by the nonactin-K complexes at the PE side. $\phi_{\text{GDO}} + \Delta\phi$ is the height of the barrier 'seen' by the nonactin-K⁺ complexes at the GDO side. $\Delta\phi$ is the difference in dipole potential between both lipids. n , the fractional distance to the corner, is 0.32 ± 0.03 . a, The expected barrier profile obtained by superposition of the dipole potential profiles for a GDO and PE monolayer. $\phi_{\text{PE}} = \phi_{\text{GDO}} + \Delta\phi$, and therefore there is no net flow of current at 0 potential. b, When a positive voltage V is applied, ϕ_{PE} is reduced by nV and the current flux J is proportional to $\exp(neV/kT)$ for large potentials ($V > 75$ mV). c, The negative potential applied exactly compensates for the differences in dipole potential. By using similar triangles it is easily shown that $\Delta\phi = V/(1-2n)$. Here the barrier height ($\phi_{\text{GDO}} + \Delta\phi$) is reduced by $(1-n)V$ and therefore J is proportional at large voltages to $\exp[e(1-n)V/kT]$. If $V > \Delta\phi/(1-2n)$ then the height of the right hand corner of the barrier will become the current limiting step.

kinetic limitations from the formation of complexes at the membrane surface, the present method provides a way to measure such a difference directly, and does not depend on the nonactin concentration because it measures a difference in shape and not in magnitude. It also allows us to monitor

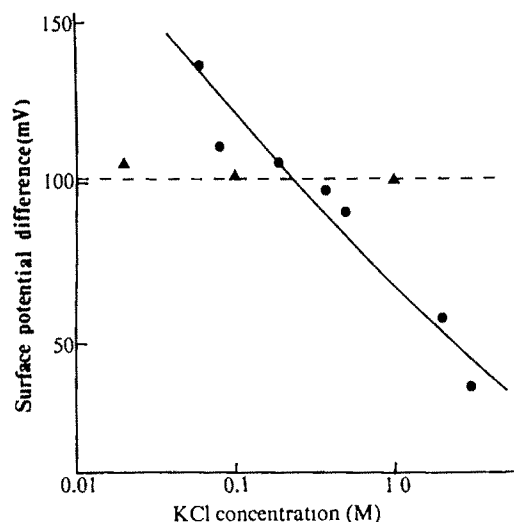


Fig. 3 Surface potential difference as a function of ionic strength. ●, Asymmetry potentials of membranes formed from one monolayer of phosphatidylserine and one of phosphatidylethanolamine. The dependence of surface potential on ionic strength is very nearly as predicted from the Gouy-Chapman equation with a surface charge density of 1 electronic charge per 75 \AA^2 (shown by the solid line). ▲, Asymmetry potentials of membranes formed from one monolayer of phosphatidylethanolamine and one of glycerol dioleate. The lack of dependence of surface potential on ionic strength is in agreement with a potential arising from dipoles rather than surface charge. Temperature $21 \pm 1^\circ \text{C}$.

changes in membrane asymmetry with time for membranes formed with two neutral lipids as long as they have a difference in dipole potential. Detection of 'flip-flop' in lipids in planar asymmetrical bilayer membranes has been restricted to membranes containing charged lipids^{16,7}. So far we have been unable to detect any lipid redistribution in membranes made of GDO and PE; this confirms other results⁸⁻⁹. It is of interest to note that we have detected no asymmetry in the current-voltage curves of membranes made of glycerolmonooleate and PE, even though the differences in dipole potential are $\sim 120 \text{ mV}$ (ref. 15). A possible explanation is that glycerolmonooleate distributes itself very rapidly between the two monolayers.

We have interpreted the asymmetry of our voltage-current curves as arising solely from differences in surface potential between two lipids. We believe this to be the case for two reasons. First, the asymmetry arising from charged lipids can be unambiguously attributed to a change in electric field across the membranes because of its dependence on ionic strength. Second, our neutral lipid results are consistent with values of surface potential derived from both monolayer and symmetric membrane studies^{10,13,15}.

As previously mentioned, lipid asymmetries are apparently present in many biological membranes. The differences in surface potential brought by the asymmetric lipid distribution can play an important role in affecting various charged structures in the membrane, for example the gating charges in nerves. Our work provides a means of measuring asymmetric potentials in membranes of well defined composition. Our results thus enable us to estimate how lipid composition may affect all electrical functions of biological membranes.

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The effects of antipsychotics on the turnover rate of GABA and acetylcholine in rat brain nuclei

SEVERAL lines of indirect evidence indicate that the nigrostriatal and mesolimbic dopaminergic systems interact with acetylcholine (ACh)^{1,2} and γ -aminobutyric acid³ (GABA)-secreting neurones. The reciprocal relationships among these neurones have not, however, been established with precision. Nucleus caudatus and nucleus accumbens are innervated by dopaminergic axons which have their cell bodies located in substantia nigra and ventral tegmental area⁴, respectively. These nuclei contain high concentrations of ACh⁵ and GABA⁶ and a high activity of cholineacetyltransferase (CAT)⁷ and glutamic acid decarboxylase⁸ (GAD). The nucleus accumbens and nucleus caudatus are connected with globus pallidus and substantia nigra which also contain GABA⁹, ACh¹⁰ and their synthesising enzymes. Antipsychotics, including chlorpromazine, clozapine and haloperidol increase the turnover rate of dopamine (DA) in the nucleus caudatus and nucleus accumbens¹¹. Clozapine, a non-cataleptogenic antipsychotic, preferentially increases the DA turnover in the nucleus accumbens whereas chlorpromazine and haloperidol, two cataleptogenic antipsychotics show a preferential effect on the nucleus caudatus¹².

We here show that the action of clozapine, chlorpromazine and haloperidol on the turnover rates of ACh and GABA in the nucleus caudatus, nucleus accumbens, globus pallidus and substantia nigra allows for a qualitative differentiation between the biochemical effects elicited by clozapine and cataleptogenic antipsychotics. The results not only shed some light on the possible functional interdependence among GABA, ACh and DA in neuronal systems of single brain nuclei but also they provide some biochemical information which may allow us to predict a pattern of specific neurotransmitter involvement in the genesis of the cataleptogenic effects of antipsychotics.

The turnover time of ACh in nucleus caudatus, substantia nigra, globus pallidus and nucleus accumbens was

measured by infusing phosphoryl (d_4) choline ($15 \mu\text{mol kg}^{-1} \text{min}^{-1}$) at a constant rate, intravenously, for 9 min (ref. 9) and killing the rats at the end of the infusion with a high intensity microwave beam focused on the skull¹⁰. The brain was sliced into 400 μm thick slices and single brain nuclei were punched out as reported earlier⁸. Haloperidol ($10 \mu\text{mol kg}^{-1}$) clozapine ($30 \mu\text{mol kg}^{-1}$) or chlorpromazine ($20 \mu\text{mol kg}^{-1}$) were injected intraperitoneally 51 min before beginning the infusion with phosphoryl (d_4) choline. The deuterium enrichment of choline and ACh was measured by mass fragmentography in various brain nuclei^{6,9}. From these data the time needed to synthesise the ACh present in each nucleus (turnover time) was calculated¹¹. The estimation of GABA turnover time was performed by killing the rats at various times after the constant rate intravenous infusion of D-glucose uniformly labelled with ^{13}C ($50 \mu\text{mol kg}^{-1} \text{min}^{-1}$ for 10 min). Rats were killed with a microwave beam at 2, 3.5 and 5 min after the end of infusion and various brain nuclei were dissected stereomicroscopically as described above. The GABA content was measured by mass fragmentography as reported earlier¹², the turnover time of GABA was calculated from the percentage incorporation of ^{13}C into glutamate and GABA¹³. Haloperidol ($4 \mu\text{mol kg}^{-1}$, intraperitoneally) and clozapine ($30 \mu\text{mol kg}^{-1}$, intraperitoneally) were injected 40 and 20 min respectively, before the infusion with ^{13}C -glucose

Haloperidol, which is virtually devoid of anticholinergic effects¹⁴, reduced the ACh turnover time in the nucleus caudatus and nucleus accumbens, whereas clozapine, which is a potent muscarinic receptor blocker¹⁴⁻¹⁶ failed to change the turnover of ACh in these two nuclei (Table 1). The results obtained with haloperidol and chlorpromazine further support the current belief that nigrostriatal and mesolimbic dopaminergic synapses inhibit the metabolism of ACh in cholinergic postsynaptic-interneurons³. When dopaminergic receptors were blocked by these two cataleptogenic antipsychotics the metabolism of ACh in nucleus caudatus and nucleus accumbens increased (Table 1). Perhaps the decrease of ACh turnover time suggests that the activity of intrinsic cholinergic interneurons of nucleus caudatus and nucleus accumbens was increased. Our findings with ACh turnover time in the nucleus accumbens of rats are at variance with results obtained with push-pull cannulae perfusion of limbic and striatal areas of cats. These results show that cataleptogenic antipsychotics increase the efflux of ACh from striatum but not from limbic areas¹⁷. Since clozapine and haloperidol block the inhibition of striatal ACh metabolism caused by apomorphine¹⁸ it can be inferred that clozapine and haloperidol can block the postsynaptic, dopamine receptors in the nucleus caudatus¹⁸. Moreover, when clozapine was injected in rats receiving haloperidol the metabolism of striatal ACh failed to increase¹⁸. No direct explanation can be given for the different action of clozapine and cataleptogenic antipsychotics on the ACh turnover rate in the nucleus caudatus and nucleus accumbens.

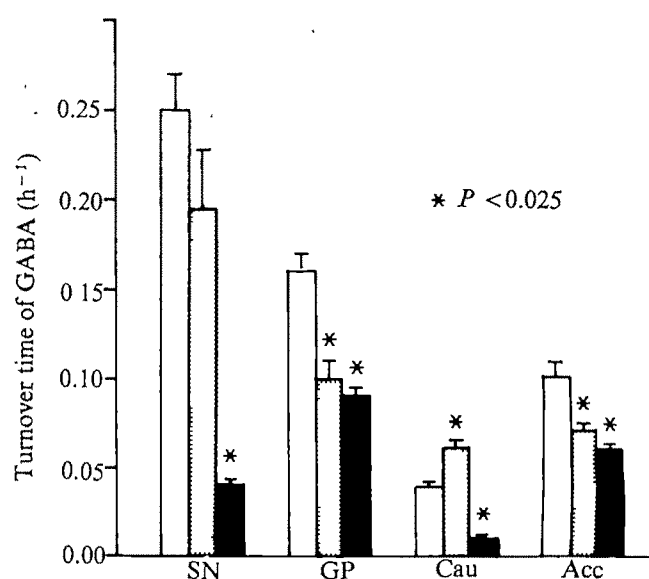


Fig. 1 Turnover time (h^{-1}) of GABA in various brain nuclei. Each bar represent an average of at least five measurements; brackets indicate s.e.m. Open bars, saline treated rats, closed bars, rats injected intraperitoneally with $30 \mu\text{mol kg}^{-1}$ of clozapine 20 min before ^{13}C glucose infusion. Dashed bars, rats treated intraperitoneally with $4 \mu\text{mol kg}^{-1}$ of haloperidol 40 min before labelling. SN, Substantia nigra, GP, globus pallidus; Cau, nucleus caudatus; Acc, nucleus accumbens.

Perhaps as proposed in earlier work¹⁸ an intrinsic positive feedback involving cholinergic synapses is operative; when this positive feedback was blocked by clozapine, the blockade of DA receptors failed to increase the ACh turnover rate in nucleus caudatus and nucleus accumbens.

Evidence is available to show that dopamine stored in dendrites of dopamine cell bodies of substantia nigra¹⁹ can be released by drugs¹⁹. In substantia nigra, the antipsychotics failed to change ACh turnover time (Table 1) suggesting that the blockade of DA receptors did not affect the ACh metabolism in substantia nigra (Table 1). However, cataleptogenic and non-cataleptogenic antipsychotics reduced the ACh metabolism in globus pallidus (Table 1).

Haloperidol and clozapine reduced the turnover time of GABA in globus pallidus and nucleus accumbens (Fig. 1). Both globus pallidus and nucleus accumbens contain high concentrations of GABA²⁰ and high GAD activity⁷. A pool of GABA-secreting interneurons, similar to that in nucleus caudatus⁴, may be present in nucleus accumbens and in globus pallidus²⁰. Since the blockade of DA receptors increased the rate of GABA metabolism in nucleus accumbens (Fig. 1), it is inferred that in this nucleus, DA receptors regulate GABA metabolism trans-synaptically. A similar suggestion can be made to explain the increase in GABA metabolism in globus pallidus because in this nucleus, which is devoid of dopaminergic terminals³, GABA may be stored in terminals from axons

Table 1 Effects of antipsychotics on acetylcholine (ACh) turnover time in various nuclei of rat brain

Brain nuclei	ACh turnover time (h^{-1})			
	Saline	Haloperidol ($10 \mu\text{mol kg}^{-1}$, i.p.)	Clozapine ($30 \mu\text{mol kg}^{-1}$, i.p.)	Chlorpromazine ($20 \mu\text{mol kg}^{-1}$, i.p.)
Substantia nigra (0.11 ± 0.018)	0.43 ± 0.07	0.31 ± 0.1	0.53 ± 0.06	0.63 ± 0.09
Globus pallidus (0.16 ± 0.023)	0.09 ± 0.03	$0.43 \pm 0.05^*$	$0.26 \pm 0.03^*$	$0.42 \pm 0.05^*$
Nucleus caudatus (0.60 ± 0.032)	0.10 ± 0.01	$0.04 \pm 0.01^*$	0.08 ± 0.01	$0.06 \pm 0.005^*$
Nucleus accumbens (0.60 ± 0.061)	0.10 ± 0.02	$0.04 \pm 0.01^*$	0.07 ± 0.01	$0.04 \pm 0.01^*$

Each value is the mean \pm s.e.m. of at least five assays. Drugs were injected 51 min before the intravenous infusion of phosphoryl (d_4) choline ($15 \mu\text{mol kg}^{-1} \text{min}^{-1}$) for 9 min. Numbers in parenthesis are the ACh concentration in $\mu\text{mol per g protein}$ (mean \pm s.e.m.; $N = 5$). i.p., Intraperitoneal.

originating in nucleus accumbens. Haloperidol and clozapine act differently on ACh or GABA turnover in all structures tested except globus pallidus where they reduce ACh metabolism (Table 1) and increase that of GABA (Fig. 1). This increase of GABA utilisation in the nucleus accumbens-pallidal pathway decreases ACh metabolism in globus pallidus probably by a trans-synaptic mechanism.

Clozapine reduced GABA turnover time in substantia nigra and striatum, whereas haloperidol failed to change GABA metabolism in substantia nigra but decreased that of striatal GABA (Fig. 1). Since substantia nigra receives GABA terminals from nucleus caudatus²⁰⁻²³, the increase in the metabolism of GABA elicited by clozapine in substantia nigra and nucleus caudatus may be interrelated. At this time we can only suggest the possibility that muscarinic receptors regulate transmitter metabolism in intrinsic GABA interneurons of striatum and in the strio-nigra GABA neurones.

Indeed a precise interpretation for the mechanisms that are operative in contributing to the difference between the effects of haloperidol, chlorpromazine and clozapine on GABA and ACh metabolism in basal ganglia awaits further investigation; however, it is important to know that an enhanced metabolism of GABA in striatum and substantia nigra is elicited by clozapine, an antipsychotic which blocks DA receptors like haloperidol and chlorpromazine but unlike these two drugs, fails to cause tardive dyskinesias or extrapyramidal side effects^{24,25,26}.

In conclusion, the cataleptogenic antipsychotics decrease the ACh turnover time in nucleus caudatus and nucleus accumbens whereas clozapine does not; the latter decreases the turnover time of GABA in substantia nigra and nucleus caudatus whereas haloperidol does not. One might speculate on the possibility that antimuscarinic and GABA mimetic actions help to limit the cataleptogenic actions of antipsychotics; in contrast a reduction of ACh and an increase of GABA metabolism in globus pallidus express a characteristic change in striatal output elicited by cataleptogenic and non-cataleptogenic antipsychotics. Since globus pallidus includes an important output from nucleus caudatus and nucleus accumbens one might infer that the changes in ACh and GABA metabolism are biochemical responses related to the antipsychotic action which are independent from the extrapyramidal side effects.

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Stimulation of pontine reticular formation suppresses firing of serotonergic neurones in the dorsal raphe

THERE is ample evidence¹⁻¹⁰ that both the pontine reticular formation (PRF; including the nucleus reticularis pontis oralis and caudalis in the terminology of Olszewski and Baxter¹¹ or the gigantocellular tegmental field in Berman's¹² terminology) and the brainstem raphe nuclei are involved in sleep processes. We report here that PRF-stimulation suppresses firing in raphe neurones. These results have implications which bear directly on current concepts about the cellular mechanisms involved in the control of the sleep cycle.

Evidence from lesion¹⁻³ and stimulation⁴⁻⁵ studies suggests that the PRF is important in the generation of rapid eye movement (REM) or desynchronised sleep (DS). Hobson *et al.*⁶⁻⁸ have found that cells in the PRF selectively increased their firing just before the onset of DS and maintained their high activity throughout the whole DS episode. On the other hand, McGinty and Harper⁹ reported that cells in the dorsal raphe nucleus (DRN) ceased their firing before the occurrence of pontogeniculooccipital (PGO) waves and their activity remained low during the ensuing DS. The occurrence of PGO waves is thought to be inhibited or 'gated' by the release of 5-hydroxytryptamine (5-HT)^{13,14}. It is possible that immediately preceding the onset of and during the DS episode, cells in the PRF exert an inhibitory influence on cells containing 5-HT in the DRN which, in turn, release the gate or disinhibit the PGO wave activity. Anatomical studies using Golgi¹⁵, Nauta¹⁶, autoradiography¹⁷ and horseradish peroxidase¹⁸ techniques have indicated the presence of projections from the PRF to midbrain raphe nuclei. We have also presented pharmacological evidence¹⁹ suggesting the existence of an inhibitory pathway from the PRF to the DRN. The purpose of this study was to investigate the influence of electrical stimulation of the PRF on 5-HT cells in the DRN and to explore the possible neurotransmitters which might mediate these effects.

Under chloral hydrate anaesthesia (400 mg kg⁻¹, intraperitoneally), 20 male Sprague-Dawley rats (200-300 g) were implanted with concentric stimulating electrodes in at least one, in most cases two, of the following structures: (1) the ventromedial PRF [posterior 1 mm (to lambda); lateral 0.7 mm; horizontal -8 mm (below skull surface)]; (2) the locus coeruleus (LC; P 1 mm; L 1.1 mm; H -6 mm); and (3) the nucleus trigemini principalis (NprV; P 1 mm; L2 to 2.5 mm; H -6.5 mm). Stimulation of the latter two structures served as controls. Electrodes were positioned at an angle of 10° to the vertical so that there was enough space for lowering the recording micropipettes. Micropipettes filled with 2 M NaCl saturated with fast green FCF²⁰ were lowered through a burr hole into the DRN (A 0.5 mm; L 0 mm; H -5.5 to -7 mm). Methods for differential recording of single unit activity during electrical stimulation, drug administration, histological localisation and physiological data analysis have already been described in detail^{19,20}. 5-HT cells in the DRN were tentatively identified by their wave form (that is, a predominant positive then a negative wave) and by their slow, regular spontaneous dis-

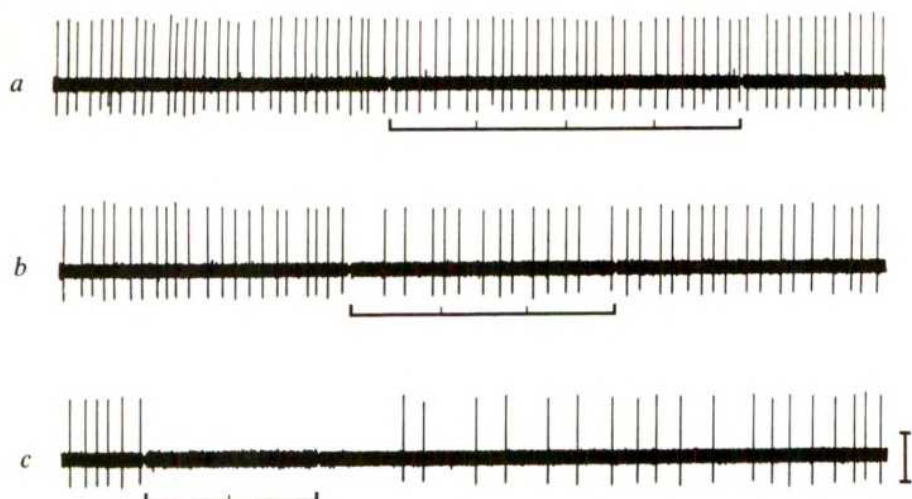


Fig. 1 Comparison between the responses of a typical 5-HT cell to PRF-stimulation and control electrode stimulation. This experiment was carried out in a 6-OHDA pretreated (8 d) rat to rule out the involvement of the LC and other adrenergic systems. We gave an intraperitoneal injection of chlorimipramine (1 mg kg^{-1}) to protect the 5-HT system before the intraventricular injection of $200 \mu\text{g}$ of 6-OHDA in $20 \mu\text{l}$ of ascorbic acid solution (1 mg ml^{-1}). *a-c*, Continuous oscillographic records of cellular discharge. The line under the oscillographic record indicates the stimulation period. Each division on the line is 10 s . In *a*, there was no suppression following the stimulation of the control electrode (10 Hz , 0.1 mA , 1 ms pulsewidth) which was located in the NprV. In *b*, PRF-stimulation at 0.05 mA (10 Hz , 1 ms) resulted in a slowing of the firing. In *c*, PRF-stimulation at 0.1 mA (10 Hz , 1 ms) produced a total suppression of cellular activity. Calibration: 0.5 mV . Positivity is upward.

charge rates ($0.5\text{--}2.5 \text{ spikes s}^{-1}$). This firing pattern has been demonstrated by combined single cell recording and fluorescence histochemical methods²² to be characteristic for 5-HT-containing cells but not for non-5-HT-containing cells in the adjacent central grey or reticular formation. Recently, the identification of the slow, regular units in the DRN as 5-HT neurones has been confirmed electrophysiologically: electrical stimulation of the ascending 5-HT axonal pathway in the ventromedial tegmentum of the anterior midbrain produces antidromic responses only in the type of unit described above (ref. 21 and R.Y.W. and G.K.A., in preparation).

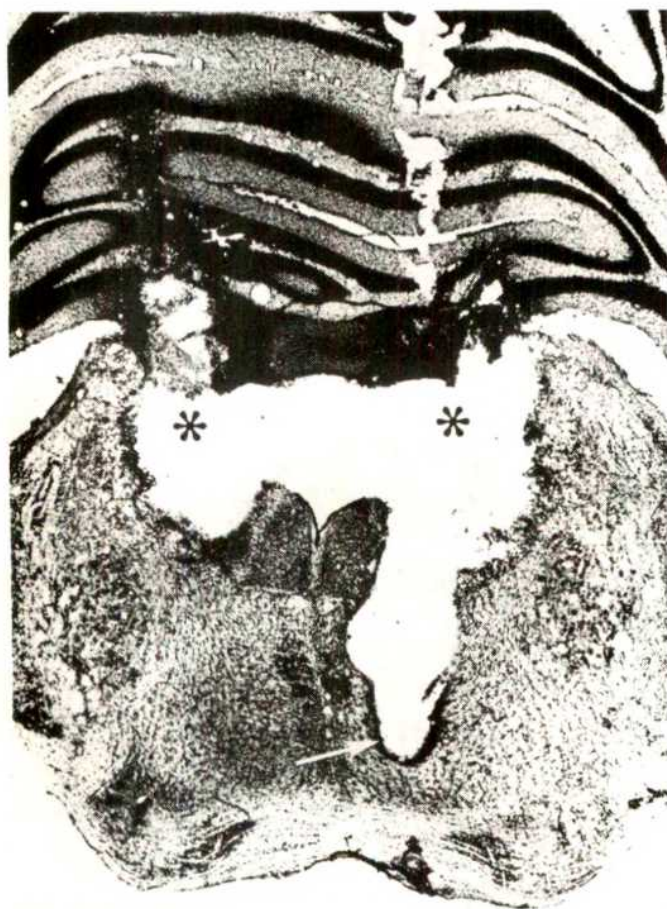


Fig. 2 Photomicrograph of a coronal section of the brain stained with cresyl violet showing that in a bilateral LC(*)-lesioned rat, the tract of the PRF stimulating electrode (arrow) is in the nucleus reticularis pontis caudalis.

Electrical stimulation of the ventromedial PRF markedly suppressed spontaneous firing of 5-HT cells in the DRN; in many cases suppression of firing was produced by PRF-stimulation at relatively low frequencies and currents. The term 'suppression' is used in preference to 'inhibition' because we cannot rule out the possibility that the depressant effects of PRF-stimulation on DRN cells are due (indirectly) to disfacilitation. The degree of suppression of the firing of DRN cells was proportional to the strength of the stimulus applied to the PRF (Fig. 1*b* and *c*). Repeated stimulation at 1 Hz ($0.05\text{--}0.2 \text{ mA}$, pulsewidth 1 ms) resulted in a poststimulus period of total suppression lasting $50\text{--}300 \text{ ms}$ in 66 of 68 (97%) typical 5-HT cells. Of these 66 cells, 7 showed an initial excitation preceding the period of suppression. None of the cells ($N = 4$) which lacked the characteristic firing pattern of 5-HT neurones was affected by the stimulation. Most of the 5-HT cells responded to the PRF-stimulation with a short latency of suppression (range, $0\text{--}66 \text{ ms}$; mode 0 ms ; median, 8 ms ; mean $\pm \text{s.e.}$, $10.0 \pm 11.5 \text{ ms}$). By 0 latency ($N = 14$) we mean that the onset of the suppression was so rapid that no spike occurred in between onsets of the stimulation and the suppression of firing. Although the short latency of this effect suggests the existence of a monosynaptic PRF-DRN pathway, a polysynaptic pathway cannot be ruled out by our data. The mechanism of PRF-induced suppression of firing cannot be attributed to recurrent inhibition because none of the recorded raphe cells showed signs of antidromic activation (that is, invariant latency, faithful response to high rate of stimulation and collision to orthodromic spikes). The absence of antidromic responses might be due to the fact that there are only scattered 5-HT terminals in the reticular formation²³.

In four experiments, stimulating electrodes were put into different regions of the anterior medullary reticular formation. In these rats, 5-HT cells ($N = 6$) in the DRN were not suppressed by the stimulation. This suggests that the effects of PRF stimulation are mediated by cells which originate in this

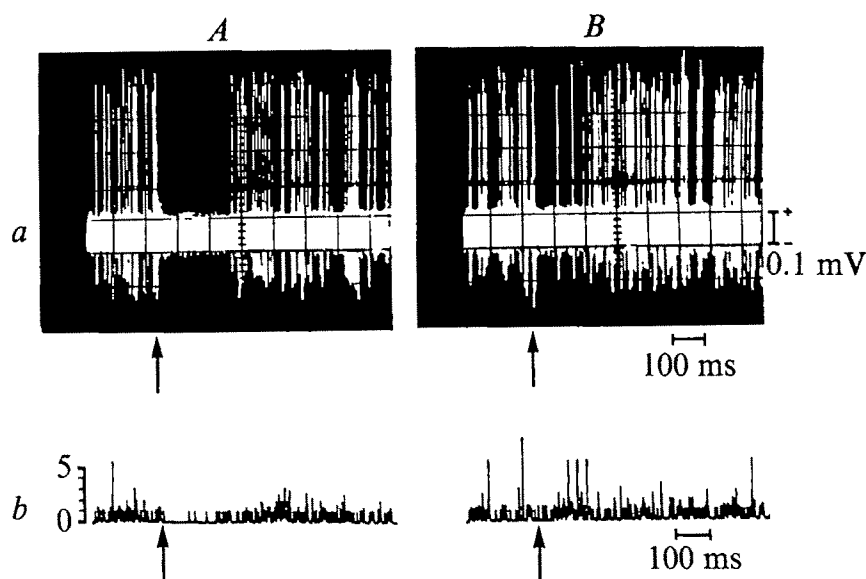
Table 1 Comparison between the response of 5-HT cells to PRF stimulation and to control electrode stimulation

Electrode location	No. of rats	No. of cells suppressed	No. of cells not suppressed	% Suppressed
PRF	18	66	2	97%
LC*	4	4	15	21%
NprV*	9	3	19	14%

PRF, pontine reticular formation; LC, locus coeruleus; NprV, nucleus trigemini principalis.

*Compared to PRF stimulation, the proportion of 5-HT cells suppressed by stimulation of control electrodes is significantly lower ($P < 0.001$, χ^2 test with Yate's correction). The difference between the LC and the NprV groups is not significant.

Fig. 3 PRF-induced suppression of firing of 5-HT neurones and their blockade by intravenous injection of the GABA antagonist picrotoxin. *a* and *b* are two different 5-HT cells from two rats in separate experiments. Activity of neurones was displayed either directly on a storage oscilloscope (cell *a*) or in the form of a peri-stimulus time histogram (cell *b*). Every picture and histogram consists of at least 150 sweeps. The latency of suppression was determined by measuring the time elapsed between the onset of stimulation and the last spike preceding the onset of suppression. Arrows indicate the onset of electrical stimulation. In column *A*, before administration of picrotoxin, PRF-stimulation had a marked effect on 5-HT neurones. In column *B*, after the intravenous injection of picrotoxin, PRF-induced suppression was reduced in cell *a* (2.5 mg kg^{-1}) and was totally blocked in cell *b* (6 mg kg^{-1}). Vertical counts in cell *b* represent number of spikes in each address.



area rather than ascending fibres. This agrees with the anatomical study of Nauta¹⁶ who has shown that the segment of the ascending component of Forel's tractus fascicularum which projects to the midbrain raphe area originates at a more rostral tegmental level rather than from the medulla.

When the stimulating electrode was located either in the LC or in the NprV, there were considerably fewer 5-HT cells suppressed by the stimulation. Table 1 lists the number of 5-HT cells suppressed by stimulation of the PRF, LC and NprV. Compared to PRF-stimulation, the proportion of 5-HT cells suppressed by stimulation of either the LC or the NprV is significantly smaller ($P < 0.001$, χ^2 test); latencies of suppression produced by stimulation of control electrodes (combined LC and NprV group) were longer ($16.5 \pm 15.9 \text{ ms}$ against $10.0 \pm 11.5 \text{ ms}$, $P > 0.05$, two-tailed t test); and current thresholds for control electrodes to elicit suppression of firing on 5-HT cells were much higher ($0.27 \pm 0.06 \text{ mA}$ against $0.09 \pm 0.04 \text{ mA}$, $P < 0.001$, two-tailed t test; Fig. 1a).

To demonstrate further that stimulation of the LC does not contribute materially to PRF-induced suppression, we bilaterally destroyed the LC before the stimulation and recording experiments. Using either radio-frequency lesions or chemical lesions with intraventricular injection of 6-hydroxydopamine (6-OHDA), we have consistently destroyed the LC¹⁹. The extent of the LC lesions was verified by either routine histological methods (Fig. 2) or the Falck-Hillarp type fluorescence histochemical method. As can be seen from Fig. 2, the radio frequency lesion destroyed the dorsolateral part of the PRF as well as the LC. In these LC (and dorsolateral PRF)-lesioned rats ($N = 6$) PRF-induced suppression of 5-HT cells was undiminished; all 15 5-HT cells were readily inhibited by ventromedial PRF-stimulation (Fig. 1b and c).

In an attempt to explore the possible transmitters involved in the PRF-induced suppression, we compared the PRF-induced effect on 5-HT cells both before and after the intravenous administration of the γ -aminobutyric acid (GABA) antagonist picrotoxin or the glycine antagonist strychnine. We found that picrotoxin reduced markedly the PRF-induced suppression of 5-HT cells ($N = 6$). Figure 3 is a representative example showing that picrotoxin reduced PRF-induced suppression at a dose of 2.5 mg kg^{-1} and totally blocked the suppression at a dose of 6 mg kg^{-1} . In contrast, picrotoxin at these dose levels, failed to block the recurrent inhibition of DRN cells produced by stimulation of the ascending 5-HT pathway (R.Y.W. and G.K.A., in preparation). Strychnine ($0.4\text{--}1 \text{ mg kg}^{-1}$, intravenously) had little or no effect on PRF-induced suppression (8 cells). These results are suggestive of a possible role for GABA in mediating the effect produced by PRF-stimulation and are consistent with our previous finding

that 5-HT cells in the DRN are inhibited by local, iontophoretic application of GABA and that this inhibition is blocked by picrotoxin¹⁹.

In conclusion, our data indicate that stimulation of the PRF markedly suppresses the activity of 5-HT cells in the DRN and that this effect might be mediated through a PRF-DRN GABAergic pathway. These results support the hypothesis that immediately preceding the onset of and continuing throughout the DS episode, cells in the PRF, which are known to increase their firing at this time⁶⁻⁸, exert a powerful, inhibitory influence on 5-HT cells in the DRN. On the other hand, we conclude that the LC has a minor and possibly indirect influence on 5-HT cells in the DRN because: (1) stimulation of the LC (which consists of cells containing noradrenaline) does not have a marked inhibitory effect on 5-HT cells in the DRN; and (2) the microiontophoretic application of noradrenaline directly on to 5-HT cells does not consistently produce inhibition^{19,24}. Experiments are now being conducted on behaving rats to eliminate the possible influence of anaesthesia on the response of DRN cells to PRF stimulation. *Note added in proof:* A recent abstract²⁵ agrees with our result that stimulation of LC has a minor and possibly indirect influence on 5-HT cells in the DRN.

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Decrease of acetylcholine receptor synthesis in muscle cultures by electrical stimulation

MUSCLE activity has been shown to be a central factor in controlling the level of extrajunctional acetylcholine receptors (AChR): inactivity of muscle fibres causes an increase in the number of AChR and electrically stimulated activity causes a decrease. We investigated the mechanism by which this activity regulates the level of AChR in muscle fibres that have differentiated in cell culture. Our results suggest that electrical stimulation decreases the AChR level by decreasing the synthesis of the receptors rather than by degrading or inactivating them.

In normally innervated muscle, AChR are located at the endplate. After surgical denervation, however, there is a great increase in the number of AChR in the region outside the endplate¹. A clear indication that muscle activity plays a part in controlling the extrajunctional AChR came from the experiments in which an electrically stimulated contraction of denervated muscle suppressed the spread of

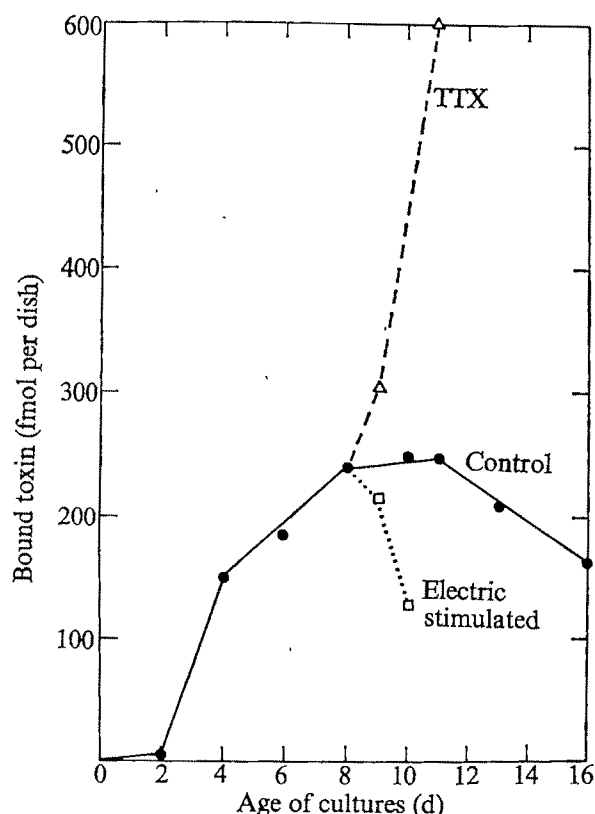


Fig. 1 Time course of ¹²⁵I- α -BuTX binding by chick muscle cultures treated with electrical stimulation (\square), or 0.5 μ g ml⁻¹ TTX (Δ). Each point represents the mean of three replicates. The electrical stimulation of the muscle was achieved through a pair of platinum electrodes immersed in the culture medium and fastened 25 mm apart, to a cover glass of the 30 mm dish. Six-second trains of 1 ms square-wave pulses with an interval between pulses of 200 ms were delivered to each dish every minute. Pulse polarity was alternated for each train to prevent polarisation. The voltage was approximately 20 V. Growing of the muscle cells and the determination of the amount of toxin bound were as described before¹⁰.

Table 1 Effect of cycloheximide on AChR

	AChR (fmol per dish)	Half life (h)
Control	219 \pm 20	28 \pm 3
Cycloheximide (20 μ g ml ⁻¹)	231 \pm 16	267 \pm 22
Stimulated	123 \pm 4	26 \pm 2
Stimulated + cycloheximide	224 \pm 14	276 \pm 26

10-d-old chick muscle cultures were treated for 48 h as indicated, then the amount of toxin bound, which reflects the amount of AChR, was measured. To measure the rate of AChR degradation, 14-d-old muscle cells were first pulsed with ¹²⁵I- α -BuTX then the cells were treated as indicated for 3 d. Half life of AChR was determined from the decline of radioactivity bound in the culture dish. Each value is the average of three replicates \pm s.d.

AChR^{2,3}. Furthermore, it was reported⁴⁻⁷ that electrical stimulation of denervated muscle reduced the supersensitivity to ACh.

The reduction in the amount of AChR following electrical stimulation could be a result of: (1) inhibition of AChR synthesis, (2) increase in AChR degradation, (3) inactivation of extrajunctional AChR. To distinguish between these possibilities we used muscle cells grown *in vitro*. Such muscle fibres were found to produce AChR⁸ and also to develop ACh supersensitivity in response to tetrodotoxin (TTX) application, which inhibits their spontaneous activity^{9,10}. When muscle cultures were stimulated electrically, the amount of AChR, as measured by labelled α -bungarotoxin (α -BuTX) binding, was reduced considerably, compared to the control or to the TTX-treated cells (Fig. 1).

The above-mentioned difference between stimulated cultures and those of the control could be explained by a different turnover rate of AChR. Such a difference in the turnover rate of junctional and extra-junctional receptors has been reported^{5,11,12}. To test this possibility, muscle cultures were first pulsed with ¹²⁵I- α -BuTX, then electrically stimulated and the rate of toxin loss was measured (Table 1). If we assume that the toxin loss reflects intracellular degradation of toxin-receptor complex¹³, then the results show that the various treatments had very little effect on AChR degradation rate. (The half life fell within a 26-28 h range.) When protein synthesis was inhibited in the electrically stimulated cultures by cycloheximide, no decrease in the amount of AChR was observed (Table 1). This result is in contrast to the above-mentioned decrease in AChR resulting from electrical stimulation in the absence of protein synthesis inhibitor. This effect of cycloheximide might be explained, as has been suggested^{11,13}, by an inhibition of receptor degradation (Table 1). The fact that there is no decrease in the amount of AChR in the presence of the inhibitor suggests that the electrical stimulation *per se* does not inactivate the receptors.

To measure the effect of electrical stimulation on receptor synthesis, the existing cholinergic receptors were saturated with unlabelled α -BuTX. Then the rate of appearance of new receptors was measured by binding of radioactive α -BuTX after various periods of stimulation. It was reproducibly found that the stimulated cultures bound much less ¹²⁵I- α -BuTX than did the control, or the TTX-treated cultures (Fig. 2). These experiments suggest that the decrease in α -BuTX binding as a consequence of electrical stimulation is due to a partial suppression in receptor synthesis rather than an inactivation of existing AChR, or an increase in the rate of their turnover.

Walker and Wilson¹⁴ have reported that the electrical stimulation of cultured muscle cells caused a 30-90% decrease in acetylcholinesterase (AChE) activity. Although we used the same technique for measuring the enzyme activity, our results differed considerably; in our case the decrease in AChR after electrical stimulation was accompanied by no significant change in AChE activity. Since cell injury might be the cause of the decreased AChE activity,

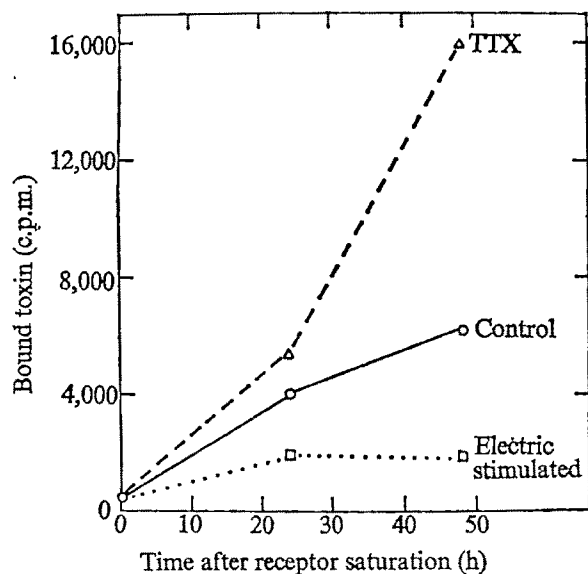


Fig. 2 Kinetics of ^{125}I - α -BuTX binding after saturation of AChR with cold toxin. The appearance of new receptors in 9-d-old muscle cultures was followed with labelled α -BuTX during treatment with electrical stimulation (\square), or TTX (Δ).

we monitored possible cell damage in our experiments by measuring the activity of creatine phosphokinase (CPK), which was established as an indicator for myotube proteins¹². In the experimental conditions which we chose, the electrical stimulation caused no change in CPK activity, nor was any cell damage observed (such damage was observed if the stimulation was given at a much higher frequency). Furthermore, since total protein synthesis and nucleic acid synthesis in stimulated cultures were similar to those in controls, we assume that the effect of electrical stimulation on synthesis was not a general one, but specifically affected the receptor synthesis machinery.

In conclusion, muscle fibres differentiated *in vitro* in the absence of nervous tissue have been used as a model system for the study of molecular mechanism accompanying denervation and reinnervation without the effect of a neurotrophic factor. While inactivity, like denervation, induced receptor synthesis, mechanical activity suppressed it. These results do not exclude some additional trophic influence of nerve on the muscle along with the effect of muscle usage. The existence of such an additional neurotrophic effect has recently received further support^{16,17}.

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Effect of guanine alkylation on mispairing

ALKYLATING agents, such as nitrosamines, nitrosoureas, dimethylsulphate, and so on, are known to cause mutations and cancerous growth¹⁻³. Since nitrosamines can be produced by the reaction of amines and nitrous acid, the presence of nitrites and nitrous acid in many food items as well as in the urban environment has become a source of concern^{4,5}. Recent evidence⁶ has indicated that carcinogenesis and mutagenesis bear a strong relationship to each other, although agreement is not unanimous⁷. *In vivo* alkylation of DNA occurs mainly at the N7 position of guanine (G)¹⁻³. Minor alkylation of G at the O6 and N3 positions is also known to occur⁸⁻⁹. Whether alkylation at any one of these sites is mainly responsible for mutagenesis and carcinogenesis is still an open question, although O6 alkylation seems to be favoured^{8,9-11}. We have investigated the effect of methylation at each of these three sites on the ability of G to mispair with a thymine (or uracil), which may lead to a mutation, and the mechanism involved in such mispairing, using the CNDO/2 method of calculation. The calculations are carried out for the 9-methyl purines and 1-methyl pyrimidines to simulate the bases in the DNA helix. The details of the calculations are published elsewhere¹². The magnitudes reported are particular to the geometrical parameters used here¹³. Since we were only interested in the relative effect of various G methylations, the qualitative trends observed here are expected to hold; no attempt at a full minimisation of the geometric parameters was therefore made; (a horrendous task for molecules of such sizes).

Figure 1 shows the normal guanine-cytosine (G-C) and the

Fig. 1 G-C (a) and G-U base pairs (b).

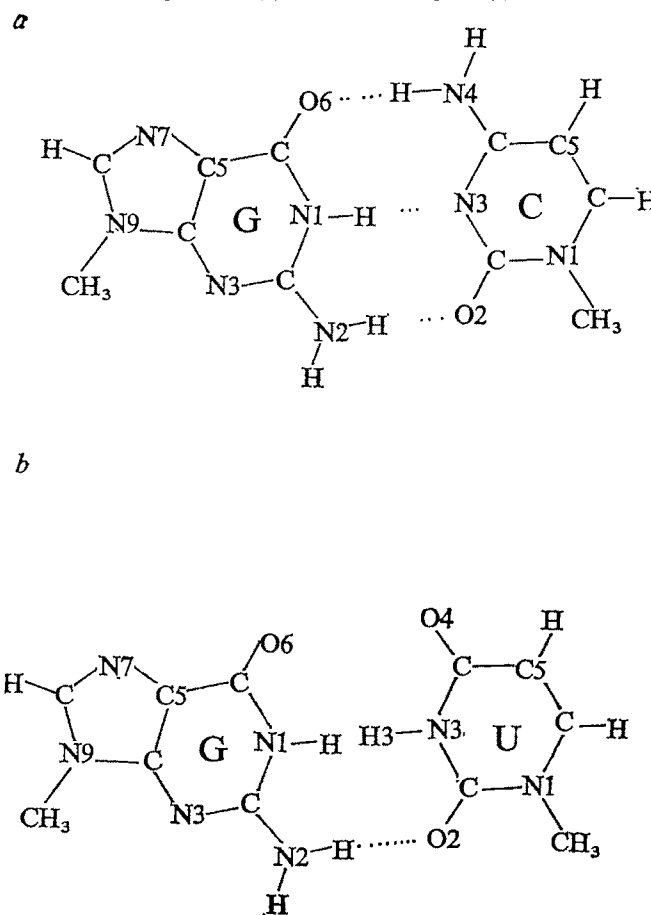


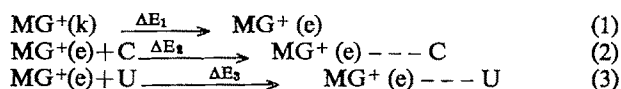
Table 1 Interaction energies (kcalorie mol⁻¹) of G and M G for the processes in mechanisms a (upper line) and c (bracketed values in lower line)

	G	N7 MG	O6 MG	N3 MeG
ΔE_1	12.70 (513.91)	13.76 (408.65)	100.33 (371.49)	-2.37 (375.90)
ΔE_2	22.06 (-12.83)	16.37 (-13.53)	14.42 (-7.03)	11.60 (-16.99)
$\Delta E_3 - \Delta E_2$	-37.34 (7.84)	-37.05 (7.77)	-44.39 (-0.70)	-33.49 (10.95)
$\Delta E_T - \Delta E_T^*$	0 (0)	1.35 (-105.33)	80.58 (-150.96)	-11.22 (-134.90)

* $\Delta E_T = (\Delta E_3 - \Delta E_2) + \Delta E_1$; $\Delta E_T^* = \Delta E_T$ for unmethylated G.

anomalous guanine-uracil (G-U) base pairs. Whereas the former base pair is stable in the present calculations by -22.64 kcalorie mol⁻¹, the latter is unstable by 20.03 kcalorie mol⁻¹. The stability of G-U can be enhanced by increasing the number of hydrogen bonds between G and U. This may be achieved by the interaction of: (a) a-G in the enol (e) form, where the H on N₁ is moved to the O6 position, with U in the keto (k) form; (b) an-U in the e form, where the H on N3 is shifted to position O4, with G(k). (We have already shown that this type of mispairing takes place in the case of 5-fluorouracil¹³, a known mutagen); (c) an ionised G, in which the H on N1 is removed, with U(k). This mechanism was proposed by Lawley and Brookes¹⁴, who found a large increase in the extent of such ionisation on alkylation at the N7 position. Mechanisms a and b follow the original explanation for mispairing proposed by Watson and Crick¹⁵.

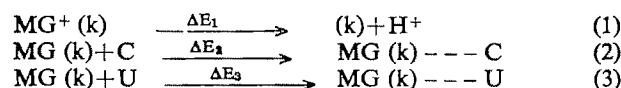
In mechanism a one must estimate the energies for each of the three equations:



where MG⁺ indicates a methylated G cation. We will now denote the specific site of alkylation by a subscript to M. Table 1 shows that the e form is favoured over the k form only in the case of M₃G⁺, and that alkylation of the N3 position is the only one that leads to a greater probability for mispairing by this mechanism than G itself.

Mechanism b is very improbable due to the very low probability of finding a U in the e form¹⁶. Nevertheless, our calculations for the competitive interaction of a G(or MG⁺) with a C(k) compared with a U(e), predict that most mispairing would occur in the case of the unalkylated G. This mechanism therefore cannot explain the increase in mutation on alkylation.

In mechanism c one must compare the energies for the following steps involving the various purine species.



These energy values are shown in brackets in Table 1. The ΔE_1 values are obtained as the difference between the energies of MG(k) and MG⁺(k) for the methylated species and of G-(k) and G(k) for the unalkylated G. To obtain reliable estimates of the overall energy change in solution for step 1, one must tackle the difficult problem of solvent interaction^{16,17} with each of the purine species as well as with the H⁺ involved in the ionisation of the purines. Nevertheless, the predicted qualitative trend of an increase in ionisation on alkylation at N7 is consistent with experimental observation¹⁴. The relative differences between the ΔE_1 values for the methylated species should still

hold upon inclusion of solvent effects because of the expectedly quite close MG⁺-solvent interaction energies of the three methylated cations. Note that the greatest extent of ionisation is predicted for M₆G⁺, and that on losing the proton it is the only species that prefers to pair with a U(k) rather than a C(k), reflected in its negative ($\Delta E_3 - \Delta E_2$) value. The total relative energies in the last row of Table 1 shows that all three types of methylation increased the extent of mispairing by this mechanism in the order: M₆G⁺ > M₂G⁺ > M₇G⁺.

The results of mechanism c are consistent with the experimental ones of Gerchman and Ludlum¹¹. These authors found that O6 methylated G mispairs to U much more than N7 methylated G or G itself; and that this greater extent of mispairing was unaffected by the presence of C as a competitor.

Our results predict that methylation at the O6 and N3 positions is more effective than at the N7 position in leading to mispaired bases and therefore to mutations and possible cancerous growth. Whereas mispairing occurs by mechanism c for O6- and N7-methylated G, for N3 methylated G it may occur by either or both mechanisms a and c. The relative importance of these two mechanisms must await experiments on the relative extent of ionisation compared with enol formation for M₃G⁺.

Our calculations indicate that methylation at the O6, N7 and N3 positions of G increases the stability of the G-C base pair by -3.80, -5.22 and -11.54 kcalorie mol⁻¹, respectively. If all other interactions within the DNA helix remain unaffected (a most unlikely situation) the methylation of G while in the helical structure would therefore help stabilise the helix against denaturation. The resulting reduction in DNA replication may be responsible for the somewhat weak chemotherapeutic action of monoalkylating agents¹⁸.

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Interferon, double-stranded RNA and mRNA degradation

INTERFERONS are glycoproteins which are synthesised in various animal cells following viral infection. They are excreted, interact with other cells and inhibit in them the replication of a broad range of viruses¹. Extracts from interferon-treated Ehrlich ascites tumour cells (S30_{INT}) differ in various biochemical characteristics from extracts from control cells (S30_C) (refs 2-4 and see also refs 5-8). We have reported recently that reovirus messenger RNAs (mRNAs) are degraded faster in reaction mixtures containing S30_{INT} than in those containing S30_C, but only if the reaction mixtures are supplemented with double-stranded (ds) RNA⁹. We have also reported¹⁰ that dsRNA promotes the phos-

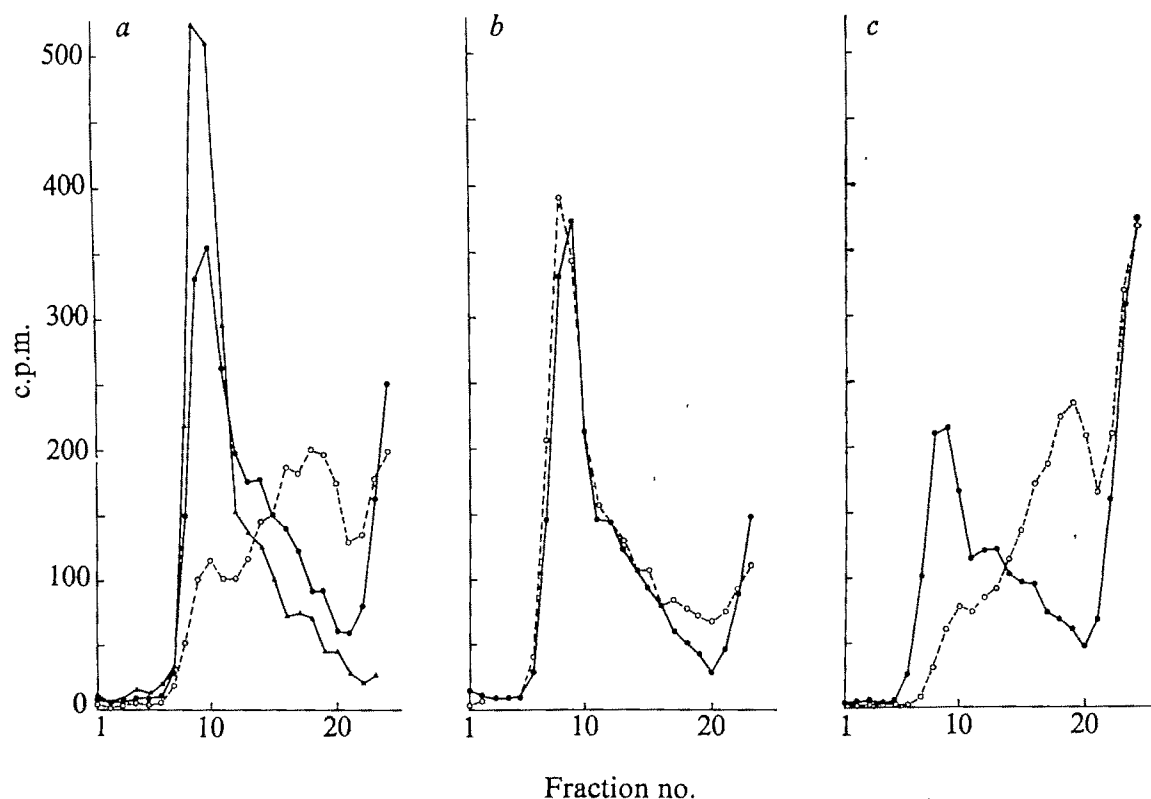


Fig. 1 The preparation of $S30_C$, $S30_{INT}$, reovirus dsRNA, 3H -labelled reovirus mRNA (enriched in medium size class mRNAs) and the procedures for the reovirus mRNA degradation assay have been described before⁹. The reaction mixtures included $30 A_{260} ml^{-1}$ of $S30$ which had been passed through Sephadex G-25, $5 \mu g ml^{-1}$ of reovirus dsRNA, $50 \mu g ml^{-1}$ of 3H -labelled reovirus mRNA as well as all the components of an *in vitro* protein synthesising system⁹, with the following omissions: *a*, amino acids, *b*, amino acids and all the energy source (namely, ATP, GTP, CTP, phosphoenolpyruvate, creatine phosphate, creatine kinase); *c*, amino acids and all the energy source except ATP. The reaction mixtures were incubated at $30^\circ C$ for 30 min. The reactions were terminated by the addition of buffer A (0.1 M NaCl, 10 mM Tris-Cl (pH 7.5), 1 mM EDTA) containing 0.5% SDS. Proteins were removed by extraction with equal volumes of phenol. The aqueous layers (containing RNA) were layered on 12.5-ml linear sucrose gradients (7–25% w/v) in buffer A. The gradients were centrifuged at $2^\circ C$ at 39,000 r.p.m. for 12 h in the SB283 rotor in an IEC B60 ultracentrifuge. Fractions were collected and the radioactivity in each was determined. Δ , Unincubated reaction mixture with $S30_{INT}$; \bullet , reaction mixture with $S30_C$; \circ , reaction mixture with $S30_{INT}$.

phorylation by ATP of at least two proteins in $S30_{INT}$. Here we describe further characteristics of the faster degradation of reovirus mRNA in $S30_{INT}$ supplemented with dsRNA. ATP is required, in addition to dsRNA, for the acceleration of mRNA degradation in $S30_{INT}$ (ref. 11), and we show that this phenomenon can be divided into two phases. In the first phase (activation) both dsRNA and ATP, but not reovirus mRNA, are required; in the second phase (endonuclease action), in which added reovirus mRNA is degraded, it seems that neither dsRNA nor ATP has to be present.

Previously we have observed the faster degradation of reovirus mRNA in the presence of dsRNA in reaction mixtures containing $S30_{INT}$ as well as all components used for *in vitro* protein synthesis^{9,10}. As Fig. 1*a* shows, faster degradation is also manifested in a reaction mixture not supplemented with amino acids. It is not manifested when neither amino acids nor the energy source is present in the reaction mixture (Fig. 1*b*), but it is manifested when ATP is added (Fig. 1*c*). The commercially available ATP can be replaced by a further purified ATP preparation containing less than 0.1% ADP as the only impurity detected by chromatography and ultraviolet absorption.

The existence of two phases in the phenomenon of faster reovirus mRNA degradation in $S30_{INT}$ is revealed in the experiments shown in Figs 2 and 3. The addition to the reaction mixture of the various components, namely dsRNA, ATP, RNase III (an enzyme hydrolysing dsRNA, but not single-stranded RNA)^{12,13}, glucose and hexokinase (transforming ATP to ADP), and reovirus mRNA, was staggered in these experiments. The results in Fig. 2*a* and *b*

verify that dsRNA is required, in addition to ATP, for the faster mRNA degradation. The faster mRNA degradation is also manifested when both ATP and dsRNA are added at 0 min but dsRNA is degraded after 5 min by the addition of RNase III (Fig. 2*c*). To prove that an RNase III-treated dsRNA cannot activate $S30_{INT}$ for faster mRNA degradation it is shown that there is no enhanced mRNA degradation: (1) if dsRNA and RNase III are added simultaneously at 0 min and ATP as well as reovirus mRNA are added later (Fig. 2*d*), or (2) if dsRNA, RNase III and ATP are added together at 0 min and reovirus mRNA is added later (data not shown). These results do not rule out the possibility that segments of dsRNA might attach to protein during the 5-min incubation (in the absence of RNase III) and thus be protected from hydrolysis by RNase III added later.

The results in Fig. 3*a* and *b* verify that ATP is required, in addition to dsRNA for the faster reovirus mRNA degradation. The activated state persists even after ATP is removed by the addition of hexokinase and glucose (Fig. 3*c*). Hexokinase and glucose are effective in removing ATP since no activated state is reached if ATP, hexokinase and glucose are added simultaneously at 0 min and dsRNA and reovirus are added later (Fig. 3*d*). We have also established that a reaction mixture, which has been incubated with dsRNA and ATP (from 0 min), retains its activated state even if dsRNA is hydrolysed (by RNase III added at 5 min) and ATP is removed (by hexokinase and glucose added at 5 min) (data not shown).

These results indicate that dsRNA and ATP are needed in the activation phase but seem not to be needed in the

endonuclease action phase. The role (if any) of the dsRNA and ATP-promoted protein phosphorylation in S30_{INT} (ref. 10) in the process of activation is under investigation. It remains to be seen whether the faster degradation of reovirus mRNA in S30_{INT} is a result of increased endonuclease activity or of increased susceptibility of the reovirus mRNA to endonuclease action. An increased susceptibility of the mRNA may arise if dsRNA diminishes ribosome binding to mRNA and polysome formation more in S30_{INT} than in S30₀ (ref. 6). The hypothesis of increased mRNA susceptibility as the basis of the phenomenon is made unlikely by the finding that the protein synthesis inhibitors (sparsomycin and edeine) do not diminish the difference between S30_{INT} and S30₀ in the rate of reovirus mRNA degradation in the presence of dsRNA (to be published). The addition of dsRNA and ATP to S30_{INT} affects the rate of degradation of some RNAs much more than that of others. It accelerates the degradation of reovirus mRNAs from the large size class much more than that of those from the medium or small size classes. Furthermore, it increases the rate of degradation of an unfractionated mRNA preparation from Ehrlich ascites tumour cells much more than that of either Ehrlich ascites tumour cell ribosomal RNA or mouse globin mRNA (to be published). It may be relevant to note that

Fig. 2 The reaction mixtures included all the components as described for Fig. 1b except that 33 $\mu\text{g ml}^{-1}$ of reovirus mRNA was added only after 35 min incubation and all reactions were performed with S30_{INT} only. ATP (1 mM), reovirus dsRNA (2 $\mu\text{g ml}^{-1}$) and 25 U ml^{-1} of RNase III (a gift from Dr R. Crouch) were added as indicated at the times specified. The reaction mixtures were incubated at 30 °C for a total of 50 min. *a*, -dsRNA + ATP (0 min); *b*, + dsRNA (0 min) + ATP (0 min); *c*, + dsRNA (0 min) + ATP (0 min) + RNase III (5 min); *d*, + dsRNA (0 min) + RNase III (0 min) + ATP (30 min).

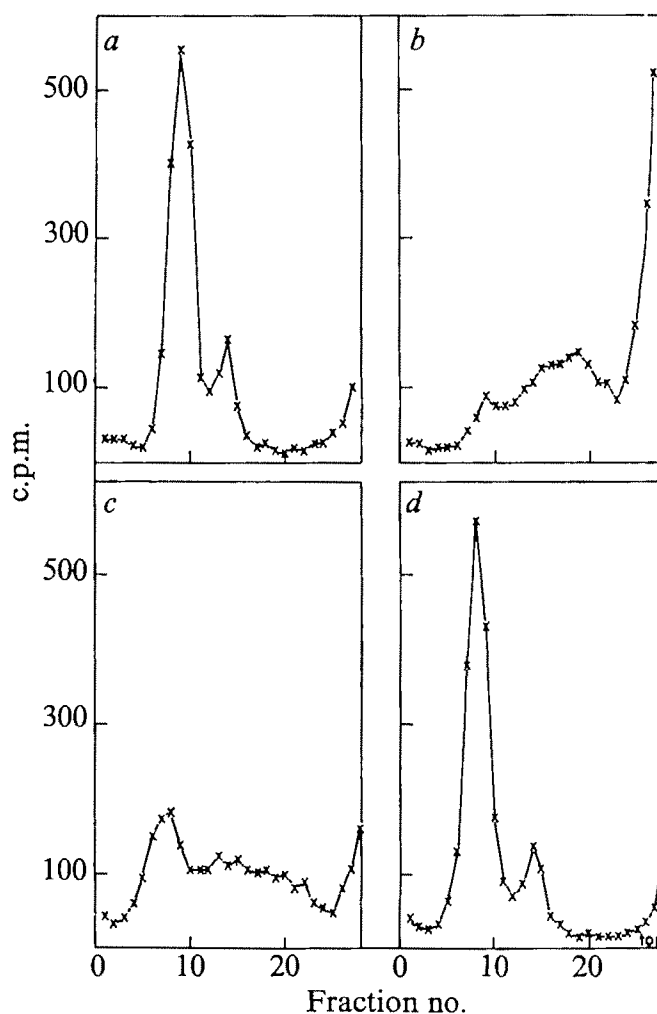
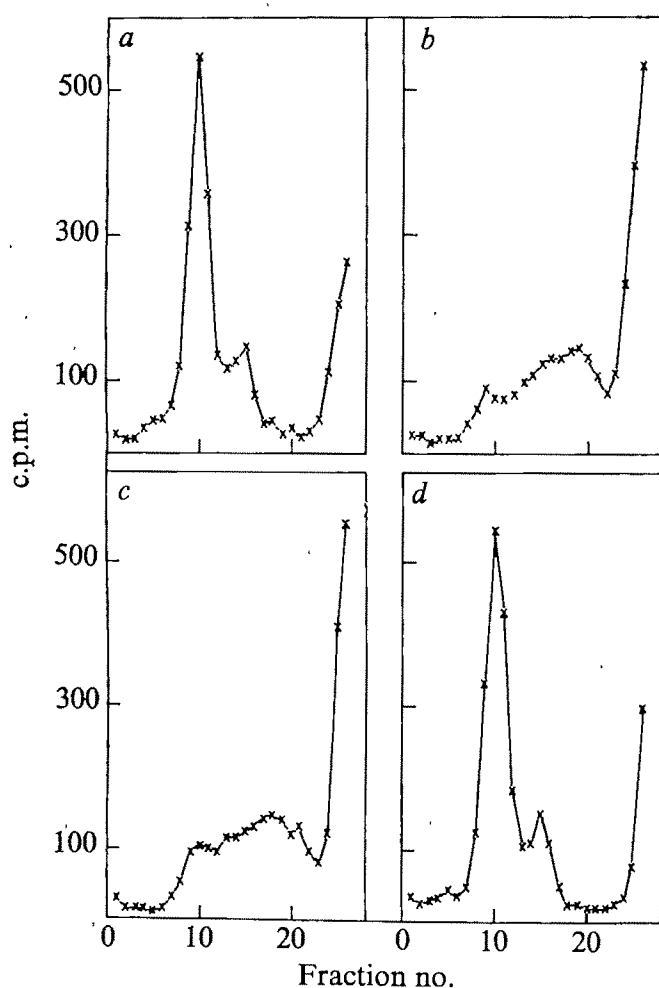


Fig. 3 The conditions were similar to Fig. 2 except that 10 mM glucose and 10 U ml^{-1} of hexokinase (Sigma) were added in *c* and *d* at 5 and 0 min, respectively. It was shown in control experiments that 10 mM glucose, or 10 U ml^{-1} of hexokinase, or 1 mM glucose-6-phosphate added individually at 0 min (together with dsRNA and ATP) did not impair the activation of the reaction mixture for faster mRNA degradation (data not shown). *a*, + dsRNA (0 min) -ATP; *b*, +dsRNA (0 min) +ATP (0 min); *c*, +dsRNA (0 min) + ATP (0 min) + glucose and hexokinase (5 min); *d*, + ATP (0 min) + glucose and hexokinase (0 min) + dsRNA (30 min).

viral RNA accumulation has been reported to be impaired in interferon-treated cells infected with different viruses¹⁴⁻¹⁷.

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- the physical state of the substrate. The enzyme from snake venom is highly active when the substrate is tightly packed in a membrane. Mammalian phospholipases cannot attack membranes; they can degrade the lecithin molecules most effectively when these are more loosely packed in micelles. All phospholipases also have some activity towards substrates in a monomeric solution. The precursors from mammalian pancreas phospholipases are also active towards the monomeric substrate molecules, but inactive to substrates in micellar form⁶. The primary structure of many of the phospholipases A₂ is known. They have a single polypeptide chain of approximately 130 amino acid residues and six or seven disulphide bridges⁷⁻¹⁰. There is a high degree of homology between all the enzymes. We report here the three-dimensional structure of one of these enzymes, prephospholipase A₂ from porcine pancreas.

Crystallisation was accomplished as follows. The enzyme was dissolved in a 0.05 M Tris-maleate buffer, pH 7.2, with 5 mM CaCl_2 . To 50 μl of this solution was added 10–15 μl of methanol. After 1 week crystals had grown to sizes up to $0.5 \times 0.5 \times 0.5$ mm. The space group of the crystal form was $P3_121$ with $a=b=69.8$ Å and $c=67.6$ Å. There was one molecule in the asymmetric unit and the solvent content

Fig. 1 Amino acid sequence of proteins with phospholipase A₂ activity. The numbering is from the porcine prephospholipase A₂ sequence. a, Porcine phospholipase A₂. Some modifications in the sequence, as given in ref. 7, will be published shortly by De Haas *et al.* b, *Bitis gabonica* phospholipase A₂ (8); c, d and e, *Naja melanoleuca* phospholipase A₂, respectively, fractions DE-I, DE-II and DE-III (9); f, *Haemachatus haemachatus* phospholipase A₂, fraction DE-I (9); g, notexin (10).

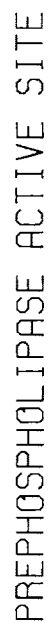


Fig. 2 Stereo plot of the main chain and disulphide bridges of porcine pancreatic prephospholipase A₂.

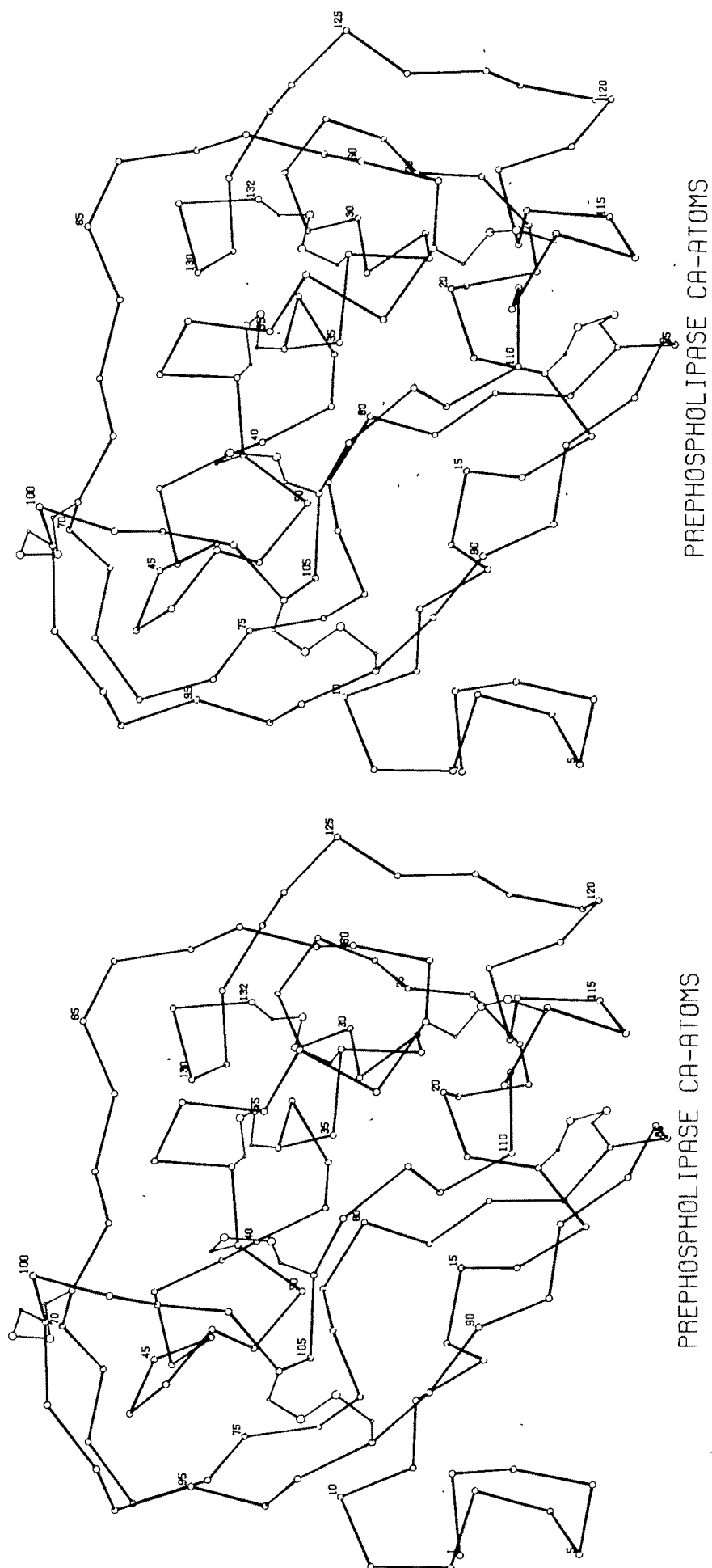


Fig. 3 Stereo plot of the active site region of porcine pancreatic prephospholipase A₂.

of the crystals was 62%. For structure determination we used one heavy atom derivative— $K_3UO_2F_6$ —with the anomalous scattering data of the uranyl atom (the SIRAS method). An electron density map was calculated to a resolution of 3 Å. This map—though of mediocre quality—could be interpreted easily with the aid of the known amino acid sequence⁷.

The polypeptide chain in the prephospholipase A_2 molecule consists of 132 amino acid residues, 14 of them involved in disulphide bridges. These seven bridges provide an internal check on the correctness of the interpretation of the electron density map, because the polypeptide chain always has to return to a bridge at a known position in the amino acid sequence.

The molecule has the shape of a rectangular box, with dimensions of approximately $25 \times 28 \times 35$ Å (Fig. 2). It contains neither regular α helices nor β -pleated sheets; only two parts are in a distorted helix conformation—residues 11–17 and 36–41. Four disulphide bridges are more or less in a plane through the middle of the molecule and three are at the surface. The N-terminal heptapeptide in prephospholipase has the sequence Glu–Glu–Gly–Ile–Ser–Ser–Arg. It shows up as a bent tail at the molecular surface. During the activation of prephospholipase by the action of trypsin the peptide bond Arg 7–Ala 8 is split. This bond is in an exposed position on the surface of the molecule.

His 55 is part of the active site of porcine (pre-)phospholipase A_2 (ref. 11). Incorporation studies, using ^{14}C -labelled *p*-bromophenacyl bromide showed that loss of enzyme activity was accompanied by the incorporation of 1.1 ^{14}C -*p*-bromophenacyl residues and by the loss of 1 histidyl residue per mol of protein. Divalent metal ions (Ca and Ba), however, protect the enzyme against this inactivation. In the electron density map, we find in the neighbourhood of His 55 a peak of density that can be interpreted as a Ca ion. It is close to the side chains of Asp 107 and Asn 57. Ca ions are also an absolute requirement for crystallisation. The crystals crack when the Ca ions are removed by addition of EDTA. When calcium binds to the protein, the spectrum in the ultraviolet region changes¹². The difference spectrum shows a perturbation of one or more tyrosine and histidine residues. One tyrosine residue, Tyr 35, is close to His 55 and the Ca-binding site (Fig. 3).

The molecular model suggests a binding mode for monomers of the substrate. It is reasonable to assume that the phosphate moiety of the substrate is bound to Arg 108 and the Ca ion. This would be similar to staphylococcal nuclease where the 5'-phosphate group of the substrate is bound specifically to the enzyme by two guanidino groups with the Ca ion 4 Å from the nearest phosphate oxygen atom¹³. In phospholipase A_2 Arg 108 is always present, except for notexin¹⁰, but the catalytic action for the latter enzyme seems to be different. The carbonyl oxygen of the ester bond to be split could well point towards the imidazole ring of His 55. Then the hydrophobic part of the lecithin molecule could fold along a highly hydrophobic surface part of the enzyme. This surface part consists of the side chains Leu 48, Cys 51–Cys 106, Thr 54, Ile 103, Phe 102 and Cys 69–Cys 99.

To predict a mechanism for the esterase activity of phospholipase we made a comparison with other ester splitting enzymes. It is well known that proteolytic enzymes have esterase as well as proteolytic activity and that the mechanism for splitting an ester bond is essentially the same as for splitting a peptide bond. X-ray crystallographic analysis of several proteolytic enzymes has provided a picture of the catalytic process in the active site.

The catalytic action of the serine enzymes starts with nucleophilic attack of the serine OH on the carbonyl carbon of the substrate^{14,15}. A tetrahedral intermediate, which has a negative charge on its carbonyl oxygen, is formed. The

Table 1 Functional groups in esterases

Enzyme	Nucleophile	Proton donor	Stabiliser
Serine enzymes	Ser OH	Imidazole H ⁺	Amide NHs
Papain	Cys S ⁻	Imidazole H ⁺	Amide NHs
Carboxypeptidase	COO ⁻ (+H ₂ O)	Tyr OH	Zinc
Thermolysin	COO ⁻ (+H ₂ O)	Imidazole H ⁺	Zinc
Phospholipase A_2	COO ⁻ (+H ₂ O)	Tyr OH	Imidazole H ⁺

tetrahedral intermediate is stabilised by amide NH groups in the active site wall. The splitting of the peptide or ester bond is accompanied by a protonation of the leaving group at the peptide NH or the ester oxygen atom. The proton donating group in the serine enzymes is the imidazole ring next to the serine, transferring a proton from the serine to the leaving group. As a result of this process the enzyme is acylated. Deacylation proceeds through similar steps, water replacing the leaving group. Thus three functions have a key role in the catalytic mechanism of the serine enzymes (Table 1): (1) the serine OH as a nucleophile; (2) the amide NH groups as stabilisers for the tetrahedral intermediate; and (3) proton donating imidazole.

From an X-ray study of papain derivatives a reasonably reliable picture has been obtained for the catalytic mechanism of this sulphhydryl protease¹⁶. It turned out to be very similar to the action of the serine enzymes. The sulphhydryl group is the nucleophile. The side chain NH₂ of Gln 19 and the main chain NH of Cys 25 contribute to the stabilising function. The imidazole ring of His 159 gives up a proton to the leaving group.

The proteolytic zinc-dependent enzymes, carboxypeptidase A_1 ¹⁷ and thermolysin¹⁸, both use the zinc ion as the stabiliser for the carbonyl oxygen. They also have the same nucleophile, which is either the carboxyl group of a Glu residue or a water molecule with its nucleophilicity increased by these carboxyl groups. The proton donating group is different for these two enzymes, being Tyr in carboxypeptidase A_1 and His in thermolysin.

A similar set of three important functional groups should be present in other enzymes with a proteolytic or ester splitting activity. In this way we could assign functions to some side chains in the active site of phospholipase A_2 . Searching for the three functions mentioned above we find Asp 56, His 55 and Tyr 35. These residues are conserved in all other phospholipases sequenced. The position of the three residues in the molecular model suggests that the Asp is the nucleophile, the imidazole ring the stabiliser and that Tyr has a proton donating function. These predictions for phospholipase A_2 should be verified, for example, by crystallographic binding studies and specific chemical modifications.

All phospholipases A_2 with known amino acid sequence show a striking homology, especially for those parts of the polypeptide chain which are in the active site region. His 55, Tyr 35, Asp 56 and Asp 107 are absolutely conserved: the conservative part 32–37 has two cysteines in S–S bridges with the main chain atoms of Tyr 35 fixed in between. The hydrophobic region shows a functional homology: only Cys 51–Cys 106 is the same in all enzymes; for the other parts a high degree of hydrophobicity is maintained, but not necessarily by identical residues. When, for example, Phe 102 is replaced by a serine residue, threonine at position 54 is always replaced by isoleucine. The most variable parts of the sequence are located on the surface of the molecule, away from the active site region.

The high activity of active phospholipase A_2 towards the micellar substrate cannot yet be explained.

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Organisation of the polypeptide chains in mammalian keratin

THE keratins are insoluble intracellular α -fibrous proteins which represent the major structural proteins of epidermis. X-ray diffraction studies of keratins in the early 1950s^{1,2} yielded a pattern since shown to be typical of intact epidermal tissue and epidermal α keratins. This pattern, which consists of a meridional arc at 5.15 Å and an equatorial reflection at 9.8 Å, was suggested to be indicative of polypeptide chains packed in a partially α -helical coiled coil configuration. Crick³ suggested that a three-stranded model was appropriate for α keratins, and although two-stranded and seven-stranded models have also been proposed, the triple α -helical coiled coil configuration has been a dominant concept in keratin structure⁴. Rudall made the initial contribution of epidermal keratin chemistry by using urea buffers to extract and characterise the α -fibrous proteins from cow snout epidermis. Matolsky subsequently showed that an α -fibrous protein, which he designated prekeratin, could also be isolated by extraction with citrate buffer, pH 2.65, and purified by isoelectric precipitation. His data indicated that the prekeratin was an α -helical, rod-like molecule with a molecular weight of 640,000 (ref. 5). Detailed studies^{6–8} have shown that epidermal keratins consist of several different polypeptide chains, and we describe here experiments which suggest how these chains may be organised in the native keratin molecule.

Prekeratin, the epidermal keratin, was prepared according to Matolsky⁵, and the sodium dodecyl sulphate (SDS)-polyacrylamide electrophoretic pattern obtained using the discontinuous system of Neville⁹ is shown in Fig. 1. The relative proportions of each of the bands were determined by scanning the gel to measure the amounts of Coomassie blue dye bound to each chain. After taking into account that the amount of dye binding is proportional to the molecular weights of the chains, it can be shown that the three major

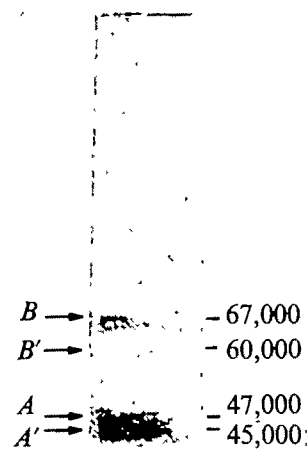


Fig. 1 SDS-polyacrylamide electrophoresis of keratin from cow snout epidermis. The gels are 7% acrylamide 2% bisacrylamide and run at pH 9.5 using the discontinuous gel system described by Neville⁹. Gels were run at 2 m per gel for 2 h at 25 °C and fixed and stained with Coomassie blue in 7.5% acetic acid (v/v) and 5% methanol (v/v). The direction of migration is downward. Samples were equilibrated in 8 M urea, 1% SDS and 1% mercaptoethanol for 1 h at 50 °C and dialysed against 1,000 volumes of upper gel buffer before loading on gels.

bands, designated B, A, A', are present in approximately equal amounts. A fourth component B' is also present but in much smaller amounts than B, A or A'. Having isolated the major polypeptide chains of keratin on DEAE cellulose as previously described¹⁰, we could perform X-ray diffraction studies on fibres pulled from solutions of purified chains and from solutions in which the chains had been recombined in the ratios shown in Table 1. Experimental detail is given

Table 1 X-ray diffraction patterns

Sample	Presence of a pattern
Prekeratin	Yes
A	No
B	No
A + A'	No
B + B'	No
A + B	No
2A + B	Yes
A + A' + B	Yes
2A' + B	Not done

Fibres (between 1 and 2 cm long) from the above solutions were prepared for X-ray diffraction in one of three ways: (1), lyophilised protein was dissolved in 80% formic acid and the gelatinous mixture was taken up and stretched between the tips of forceps; (2) solutions of protein in 0.1 M citric acid were dialysed against 0.1 M NH_4HCO_3 , pH 9.0 and the filaments that formed were picked up and stretched between forceps; (3) solutions of protein dissolved in 8 M urea containing 0.01 M Tris at pH 8.5 were dialysed against water and the filaments that formed were also picked up and stretched between forceps. The protein content of solutions of purified chains was measured by the Lowry technique before they were combined in the molar ratios shown. Fibres were mounted with a specimen-to-film distance of 1.54 cm and exposed to $\text{CuK}\alpha$ radiation ($\lambda = 1.54 \text{ Å}$) at 40 kV. Finely powdered KCl was dusted on to selected fibres before exposure and the 2.224 Å spacing was used for calibration purposes. Variations of measurements from replicate fibres was less than 0.1 Å. The presence of an α pattern was indicated by a sharp meridional arc at $5.15 \pm 0.1 \text{ Å}$ and an equatorial reflection at $9.8 \pm 0.1 \text{ Å}$.

in the legend. Purified *A* and *B* chains alone did not produce an X-ray diffraction pattern typical of α -fibrous proteins; however when these chains were combined in a ratio of 2:1 (*A* to *B*) an α X-ray pattern indistinguishable from that of either intact keratin or epidermis was obtained. This is interesting in the light of our previous descriptions of these chains which showed that *A* and *A'* and *B* and *B'* were chemically and immunologically related to one another and formed two distinct families (*A* and *B*) of polypeptide chains associated with keratins¹⁰.

Although these studies supported an unlike-chain (*AA'B* or *AA'B'*) hypothesis for the assembly of the keratin molecule, it seemed possible that a series of keratins composed only of the identical individual peptide chains (*AAA*, *A'A'A'*, *BBB* or *B'B'B'*) might exist. In the unlike-chain arrangement each of the three principal chains (*AA'B*) would be packed in a triple α -helical arrangement with a monomer molecular weight of 159,000. This unit could form dimers (molecular weight 318,000) or tetramers (molecular weight 636,000). The minor component *B'* could also form intact keratin molecules *AA'B'*, with a monomer molecular weight of 153,000 which would be present in smaller amounts and constitute a variant form. Such variant forms have been shown to exist for other structural proteins such as collagen¹¹ and fibrinogen¹².

On the other hand, if identical chains combined with themselves the following species would exist in solution: *AAA* (monomer molecular weight 135,000; dimer, 270,000); *A'A'A'* (monomer, 144,000; dimer, 288,000); *B'B'B'* (monomer, 180,000; dimer, 360,000) and *BBB* (monomer, 201,000; dimer, 402,000). It is possible that in the intact keratin molecule these species are the fundamental units from which the molecule is built.

Intact keratin molecules predicted from the unlike-chain and identical-chain hypotheses would give distinctly different SDS electrophoretic distributions as shown in Fig. 2, providing their interchain associations were not disrupted. Disruption of interchain associations was prevented by chemically crosslinking the polypeptide chains of the keratin molecule with formaldehyde using the techniques of Veiss and Drake¹³ and Nold and Cross¹⁴ for crosslinking collagen. The densitometer tracing of SDS electrophoretic patterns of crosslinked prekeratin (Fig. 3) is clearly similar to that of

Fig. 2 Predicted densitometer tracings of the SDS-polyacrylamide electrophoretic patterns of formaldehyde-crosslinked keratin. Solid line, multichain lymphoreis; broken line, single-chain hypothesis.

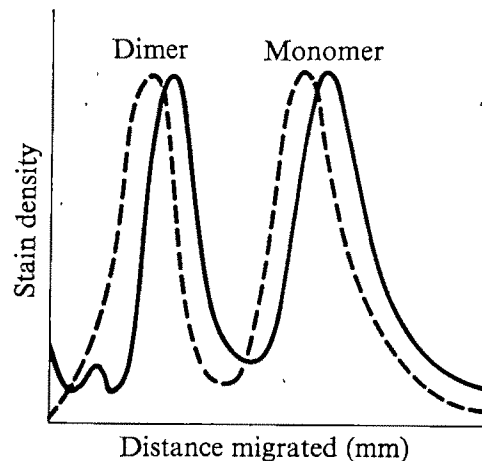
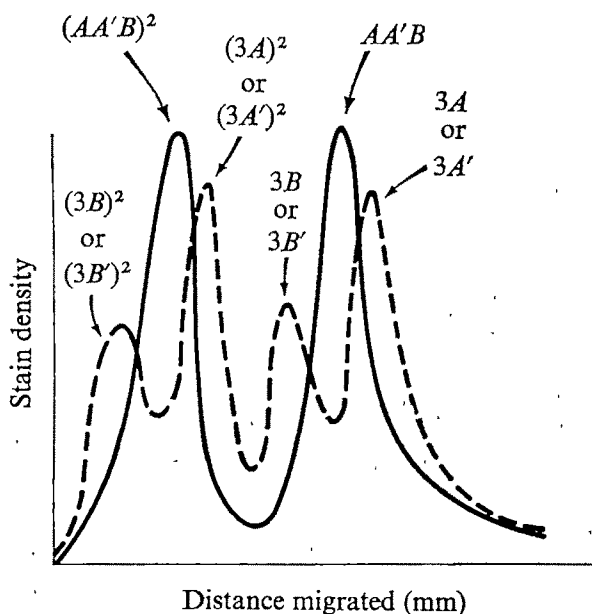


Fig. 3 The solid line represents the densitometer tracing of the SDS-polyacrylamide pattern of formaldehyde-crosslinked prekeratin and the dotted line the theoretical curve for a multichain structure. The amounts of formaldehyde were varied from 1.5 to 6.2% and the times of reaction were varied from 1 h to 3 d to obtain conditions which crosslinked all the available polypeptide chains of keratin but did not yield polymers too heavy to penetrate 5% polyacrylamide gels. The optimal conditions used in all crosslinking experiments reported were as follows. Prekeratin dissolved in 0.1 M acetic acid (1 mg ml⁻¹) was incubated with formaldehyde at a final concentration of 6.2% for 4 h at 4 °C, conditions which were shown not to crosslink solutions of single chain control proteins. After exhaustive dialysis against 0.1 N acetic acid, samples were equilibrated and run as described in Fig. 1, except that 5% acrylamide gels were used. The following molecular weight markers were used: BSA monomer (67,000) and dimer (134,000); phosphorylase A (94,000); λ globulin (150,000).

the predicted unlike-chain molecule comprised of an *A*, *A'* and *B* chain. The variant molecule (comprised of *A*, *A'* and *B'*) is too close in molecular weight to the major species to be seen as a separate peak. The molecular weights obtained for the monomer (144,000) and dimer (280,000) are approximately 10% lower than the theoretical values, which is expected as the crosslinked keratin molecules are not fully accessible to SDS. This may well be analogous to the situation with other proteins such as bovine serum albumin (BSA)¹⁵ where the folded molecule (unreduced) has a lower apparent molecular weight than the unfolded (reduced) species on SDS gels.

Our data indicate that the basic building block of the keratin molecule is composed of two *A* chains and one *B* chain. Skerrow's work¹⁷ in which he isolated a highly helical core, after tryptic digestion of keratin, which consisted of three distinct polypeptide chains, seems to support this concept. It is thought that this basic unit can readily polymerize to form the higher molecular weight units initially reported by Matoksy and is presumably the fundamental unit of the tonofilaments seen *in vivo*.

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'Sandwiched' water molecule between pyrimidine bases and intra-molecular C-H...O hydrogen bonding in 5-nitro-1-(β -D-ribose)uracil monohydrate

IN the structure of crystalline 5-nitro-1-(β -D-ribose)uracil monohydrate we have found a novel disposition of a water molecule—'sandwiched' between two parallel pyrimidine rings stacked 6.55 Å apart. The water molecule is held in its place between these rings by a short O-H...OW (2.589 Å) hydrogen bond from the carboxyl group of a uronic acid molecule belonging to an adjacent stack. We have also found that nitro groups adjacent to C-H bonds in the heterocyclic ring of nucleic acid bases apparently polarise these bonds and cause them to take part in strong hydrogen bonds to appropriate donors. The polarisation effect of the nitro group adjacent to the C-H bonds in pyrimidine is perhaps analogous to that of a positive charge adjacent to the C-H bonds in purines¹.

We have been studying the structures of a series of nitro derivatives of nucleic acid bases and nucleosides in connection with a study of radiation damage and sensitisation. To this end, we prepared crystals of 5-nitro-1-(β -D-ribose)uracil monohydrate by treating uridine with dilute nitric acid², and then slowly evaporating the resulting solution. The crystals so obtained (of $C_9H_9N_3O_9 \cdot H_2O$) are monoclinic, space group $P2_1$, with cell dimensions (at $22 \pm 3^\circ C$): $a = 8.982(1)$, $b = 10.245(1)$, $c = 6.651(1)$ Å, $\beta = 92.27(1)^\circ$, with a density of 1.74 g cm^{-3} (calculated density = 1.743 g cm^{-3}) (this is higher than usually observed for nucleosides). The crystal structure was determined from the intensities of $CuK\alpha$ X-ray 'reflections' measured by a diffractometer out to $2\theta = 165^\circ$. Multi solution³ and trial and error methods were used, and the parameters were refined by the least-squares method, in the block-diagonal approximation, to a final

Fig. 1 Structure and conformation of the molecule. Note the internal C-H...O hydrogen bonding.

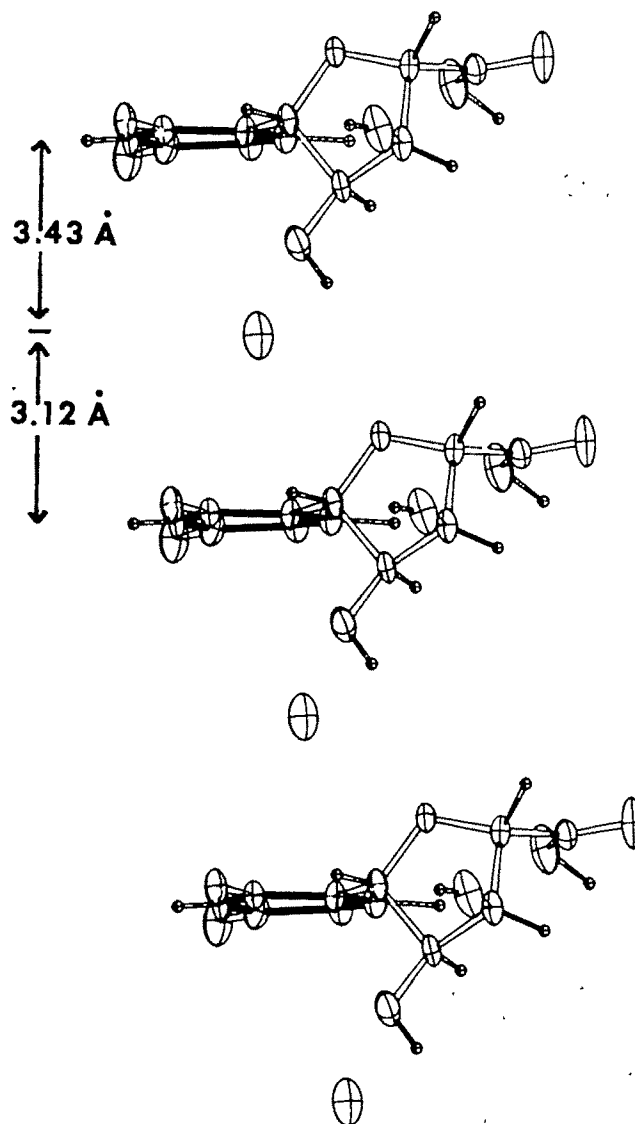
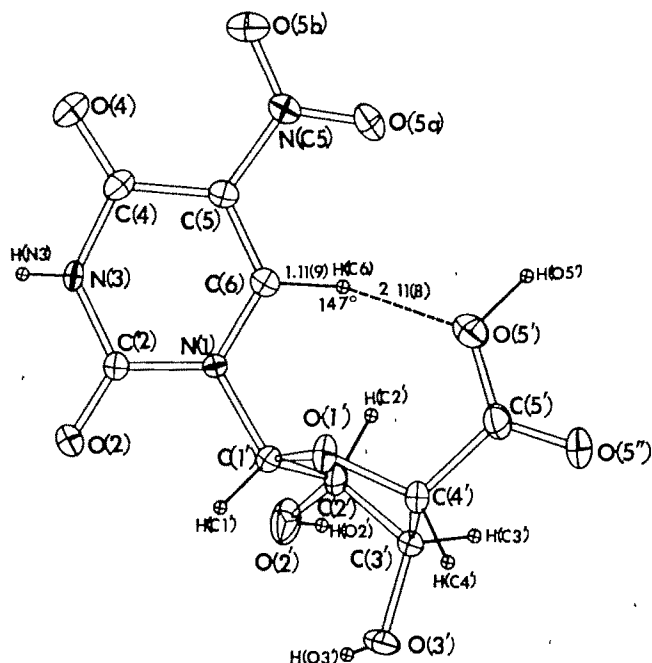


Fig. 2 The 'sandwiching' of water molecules between nucleic acid bases, as seen edgewise. The water molecule here acts as a 'spacer' keeping the bases 6.55 Å apart. The 5-nitro group has been omitted in this figure for clarity.

discrepancy factor of 0.056 for the 1,427 reflections with values of I greater than 2σ . All the hydrogen atoms, except the pair on the water molecule, were located from electron-density difference maps and their parameters were refined using individual isotropic thermal parameters.

The molecular structure and conformation are displayed in Fig. 1. The pyrimidine ring is slightly non-planar with N(1) and C(4) deviating from the least-squares plane through the other four atoms by 0.037 and 0.022 Å, respectively. The nitro group is slightly twisted away from the plane of the uracil ring; the C(4)-C(5)-N(5)-O(5b) torsion angle is 7.6° . The molecule is in the *anti* conformation ($\chi_{CN} = 53.9^\circ$), if we may extend the nomenclature for nucleosides⁴ to this molecule. The furanose ring has the C(2')-*endo*-C(3')-*exo*(²T₂) conformation, with C(2') and C(3') deviating from the plane of the other three atoms by 0.418 and -0.199 Å, respectively. The pseudorotation parameters⁵ for the five-membered ring are $P = 171^\circ$ and $\tau_m = 38^\circ$. The torsion angles O(1')-C(4')-C(5')-O(5') and C(3')-C(4')-C(5')-O(5') which describe the relative orientation of the hydroxyl with respect to O(1') and C(3') are -20.4° and 98.3° , respectively. Similar conformation of a β -D-uronic acid has been observed⁶ in thymidine 5'-carboxylic acid. In the usual nucleoside nomenclature, this conformation may be referred to as *gauche*⁺ (*g*⁺, or *gg*). But the conformational preferences of the CH₂OH and COOH groups are different and the torsion angles

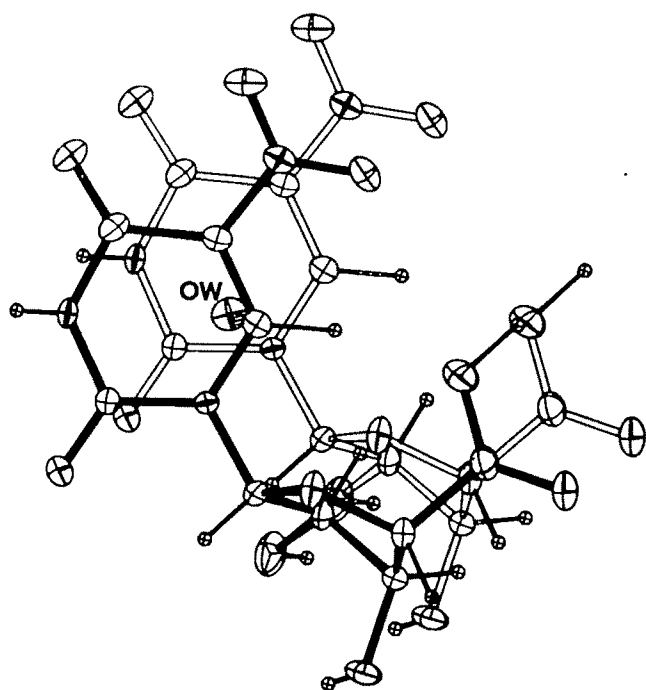


Fig. 3 Partial overlap of the nucleic acid bases and the water in between, viewed normal to the plane of the uracil ring.

are quite different from $\pm 60^\circ$. A more appropriate notation for describing this conformation may be that of Klyne and Prelog⁷; O(5') is (–) *syn*-periplanar to O(1') and (+) *anti*-clinal to C(3').

The *anti* conformation of this molecule is stabilised by an internal C(6)–H(C6) ... O(5') hydrogen bond of length 3.104 Å (Fig. 1). The role of C–H ... O (ref 8) in conferring some stability to the *anti* conformation of nucleosides and nucleotides has been pointed out by several workers^{9–11}. It is known from deuterium exchange experiments¹² and proton magnetic resonance studies¹³ in solution that the hydrogen on C(8) of purines and C(6) of pyrimidines is acidic. Our results indicate that the location of an 'electron withdrawing' nitro group adjacent to C(6)–H causes additional polarisation of the C–H bond, and thus enables the hydrogen to take part in a C(6)–H(C6) ... O(5') hydrogen bond. This polarisation effect of nitro groups in pyrimidines is analogous to that of a positive charge adjacent to C–H bonds in purines¹, as discussed by us recently. Sundaralingam^{4,10} has pointed out that nucleotides are less flexible than nucleosides. A part of this additional stability in the *anti* conformation of 5'-phosphorylated nucleotides, at least for those nucleotides that can take up a zwitterionic form, is probably due to the charge on the heterocyclic ring and the polarisation of the C(6)–H or C(8)–H bonds.

An interesting feature in the present structure is the wealth of C–H ... O hydrogen bonds and weaker interactions. All carbons in the furanose ring except C(1') take part in C–H ... O contacts in which the H ... O distances and the C–H ... O angles range from 2.27 to 2.41 Å and 130° to 163° , respectively. The uracil rings are not self-paired; N(3) is hydrogen bonded to O(5') of a neighbouring molecule (N(3)–H(3) ... O(5'), 2.855 Å; H(3) ... O(5'), 2.13 Å; N(3)–H(3) ... O(5'), 168.4°).

The most remarkable feature in this structure is the location of the water molecules; they are between successive, parallel pyrimidine bases of a stack, the members of which are related by translations along *c* and are 6.55 Å apart (Fig. 2). The water molecule is 3.43 Å from the plane of the base above and 3.12 Å from the plane of the base below, thereby forming a 'sandwich' (Fig. 3). The distances are similar to base stacking distances in nucleic acid polymers and crystals of monomers. The water

molecule is held in this location by a strong but bent hydrogen bond (O(5')–H(O5') ... OW, 2.589 Å; H(O5') ... OW, 1.71 Å; O(5')–H(O5') ... OW, 128°) from the carboxyl group of a neighbouring molecule in an adjacent stack. The shortest contacts of the water molecule are with the atoms in the pyrimidine ring below and are: O(W) ... N(1), 3.229 Å; O(W) ... C(2), 3.260 Å; O(W) ... O(1'), 3.305 Å. All other contacts are longer than 3.4 Å. Since the hydrogen atoms of the water molecule cannot be located in electron density difference maps they must be disordered. Thus, it is not possible to decide whether the O(W) ... N(1) contact is a van der Waals' contact or a weak hydrogen bond, but the stereochemistry is unfavourable for the latter.

The stacking of bases does not take place in organic solvents, but does in water; this interaction between bases has been interpreted as 'hydrophobic stacking interactions'¹⁴. It is well known that several heterocyclic dyes and polycyclic hydrocarbons bind to the bases in accord with the intercalation mechanism of Lerman¹⁵. An extensive analysis of the stacking in the solid state¹⁶ indicates that the stacking pattern in a crystal is specific and the bases stack with electronegative heteroatoms forming close contacts with adjacent aromatic systems. In view of these results, the water 'sandwich' we have found is very unusual and, as far as we know, has not been observed before. It is not clear what forces are responsible for the stability of these water-intercalated nucleic acid base stacks. In any case, our results show that such an intercalation of water molecules between nucleic acid bases is possible in certain circumstances. If so, the consequences of water intercalation in nucleic acids should be extremely important and very diverse. We shall mention only one possible example of these consequences. When there is non-complementary opposition—due either to point mutation¹⁷, or to the transient, non-complementary formation of short helices, or to enzymatic excision followed by repair of strands—the non-complementary bases may swing out and assume extrahelical conformations. In such cases, a water molecule could intercalate and act as a 'spacer', giving some stability to the nucleic acid stacks. Such intercalation of water must be very rare in view of the very limited number of mistakes that occur in nucleic acid replication. On the other hand, as our crystal structure demonstrates, such intercalation of water between nucleic acid bases cannot be ruled out. Further work is necessary to discover the conditions favourable to the formation of such hydrated polymers.

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matters arising

Stability of Lotka-Volterra systems

IN our recent paper in this journal, we claimed that global stability was a consequence of the negative-definiteness of a matrix $B = (A + A')/2$. The subsequent argument was accurate to the extent that if B is found to be negative-definite, then the system must be locally and globally stable. Since A is taken to be an arbitrary matrix, however, it is possible that A can be stable while B is not negative-definite. This means that there may be some locally stable systems that are not globally stable.

The arguments and conclusions are therefore modified as follows. Let d_i be a set of n strictly positive numbers. Introduce the Liapunov function

$$V(x) = \sum_i 2d_i[\exp(x_i) - x_i - 1]$$

which has time derivative $dV/dt = u'(DA + A'D)u$ where u is the vector with elements $[\exp(x_i) - 1]$, and D is a matrix with diagonal elements d_i and zeros elsewhere. This allows us to state the following theorem: if there exists a strictly positive diagonal matrix D such that $(DA + A'D)$ is negative-definite, the Lotka-Volterra system is simultaneously locally and globally stable.

It is not possible to say that all locally stable states are globally stable. The criterion of the previous paragraph is in general a sufficiency condition, not a necessity condition. The criterion does identify the largest known subset of locally stable states that are also globally stable. This is the strongest result available on global stability so far.

Some interesting, if enigmatic, features are worth noting. First, setting $D = I$, the unit matrix, our theorem shows that negative-definiteness of the symmetric part of the community matrix A is sufficient to ensure local and global stability. Second, if $D = N^*$, negative-definiteness of the symmetric part of the interaction matrix α which has elements a_{ij} is also sufficient to ensure local and global stability. Third, the theorem stated above identifies a subset of the possible matrices A which is also D -stable². This means that for this subset stability depends only on the interaction matrix α and is independent of the precise value of N^* , so long as all N_i^* are strictly positive. Such an identification

is in accord with intuitive feelings about the relationship between the geometry of the isoclines of the Lotka-Volterra systems and the flow in phase space.

Finally, there is a strong connection with qualitative (or sign) stability³, which involves the analysis of interaction matrices α where only the signs of the interaction elements are known. Our theorem above shows directly that Lotka-Volterra models which have sign stable interaction matrices α with strictly negative diagonal elements have globally stable dynamics.

Since submitting this manuscript the error in our original paper has independently been pointed out to us by Dr. H. I. Freedman, Department of Mathematics, University of Alberta. In addition Drs. Granero-Portat and Zanaua, Institute of Physics, Parma questioned our use of a community matrix with elements $(a_{ij}N_j^*)$; however we pointed out that such matrix has the same eigen values as a matrix with elements $(N_i^*a_{ij})$.

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¹ Tuljapurkar, S. D., and Semura, J. S., *Nature*, **257**, 388-389 (1975).

² Barnett, S., and Storey, C., *Matrix Methods in Stability Theory* (Barnes and Noble, New York, 1970).

³ Quirk, J., and Ruppert, R., *Rev Econ Studies*, **32**, 311-326 (1965).

Relatedness

ALTHOUGH the article by Dawkins and Carlisle¹ points out relevant questions regarding desertion, it also perpetuates a misconception concerning relatedness. In their example, a female with enough food to support only one infant must choose between two infants the same age, an orphaned baby brother and her own son. They conclude "Intuition points to the son but this is not necessarily correct. There are no genetic grounds for preferring either infant: the mother's relatedness to both is the same, 0.5." There is, however, a genetic difference! The degree of relatedness between two siblings is only on the average 0.5, in any particular instance it may be much less or much greater than that. Additional assumptions are required to state that behaviour is based on average relatedness. Although it could hardly be considered to prove anything, the intuitive

response to save the offspring might, in fact, indicate that nature prefers a sure thing (relatedness=0.5 as in the case of the son) to gambling (average relatedness=0.5 as in the case of the siblings). In addition, in the example they used, even the average relatedness was $\neq 0.5$. Assuming a dioecious diploid organism whose parents are not related, opposite sexed siblings are actually somewhat <50% related on average since they must necessarily have one sex determining chromosome which is not in common. Similarly, siblings of the same sex are on average somewhat >50% related.

It should be noted that the validity of their conclusions is not affected by this correction.

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¹ Dawkins, R., and Carlisle, T. R., *Nature*, **262**, 131-133 (1976).

DAWKINS REPLIES—Gibson¹ is right, both in the main point she makes and in her observation that it doesn't matter anyway. But her gambling analogy is misleading.

She thinks of the offspring as a safe bet while the sibling is a risky gamble. Yes, setting aside Gibson's trivial point about sex chromosomes, it is true that exactly 50% of the genome of a parent is inherited by a given child, while siblings share 50% of their genomes only on average. But who cares about genomes? Certainly not natural selection, at least in sexual populations². From the point of view of a single altruistic gene sitting in the body of an individual, a particular child of that individual is just as risky a prospect as a particular sibling. The gene may or may not be present in the body of the offspring, and it may or may not be present in the body of the sibling. It is a 50% gamble in both cases.

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¹ Gibson, P. A. K., *Nature*, **264**, 381 (1976).

² Dawkins, R., *The Selfish Gene* (Oxford University Press, 1976).

Fast axonal transport and amine levels

THE monoamine oxidase inhibitor, pargyline, which raises amine levels, produces a myopathy which can be prevented by previous nerve section¹. The fact that the pargyline-induced myopathy could be prevented by nerve section led Boegman and collaborators to look at effects of this drug on axonal transport. They reported that pargyline treatment caused a dramatic increase in the velocity of axonal transport², and so I decided to see what effects the reported increased velocity of transport might have on life span or composition of transported protein.

Male Sprague-Dawley rats (250–300 g) were injected intraperitoneally with the following solutions: (1) 1 ml saline for 3 d before the experiment (controls); (2) 25 mg kg⁻¹ pargyline (Parsidol, Abbott Pharmaceuticals) in 1 ml saline for 3 d; (3) 75 mg kg⁻¹ pargyline for 3 d, (4) 2.5 mg kg⁻¹ reserpine (Serpasil, Ciba) for 7 d. Injection of ³H-L-leucine into the ventral horn of the lumbosacral cord was performed as described previously¹. At intervals after injection of precursor, the sciatic nerve and ventral roots were dissected out, cut into segments, and placed in 10% TCA overnight. TCA-insoluble material was dissolved in Protosol (New England Nuclear) and counted. In a few experiments TCA-soluble activity was counted by adding Scintiverse (Fisher). In some animals whole brain, right atrium and soleus muscles were dissected out and processed according to the Falck-Hillarp technique; in others, whole brains were assayed fluorimetrically for noradrenaline (NA) and dopamine (DA).

Drug treatment was judged to be effective: treated animals showed characteristic changes in appearance and behaviour¹. There was complete loss of amine-specific fluorescence in reserpine-treated animals, and an increase in fluorescence intensity of aminergic neurones in pargyline-treated animals. Pargyline produced significant increases of 224% in whole-brain NA and 167% in whole-brain DA.

After injection of ³H-leucine, a wave of labelled protein moved along the axons of the sciatic nerve. The velocity of transport was given by the slope of the regression line on a plot of wavefront position against elapsed time. Neither reserpine nor pargyline treatment had any effect on the velocity of transport (Fig. 1). To eliminate the possibility that some labelled protein was transported at higher velocities ahead of the main wavefront, a second series of experiments was carried out.

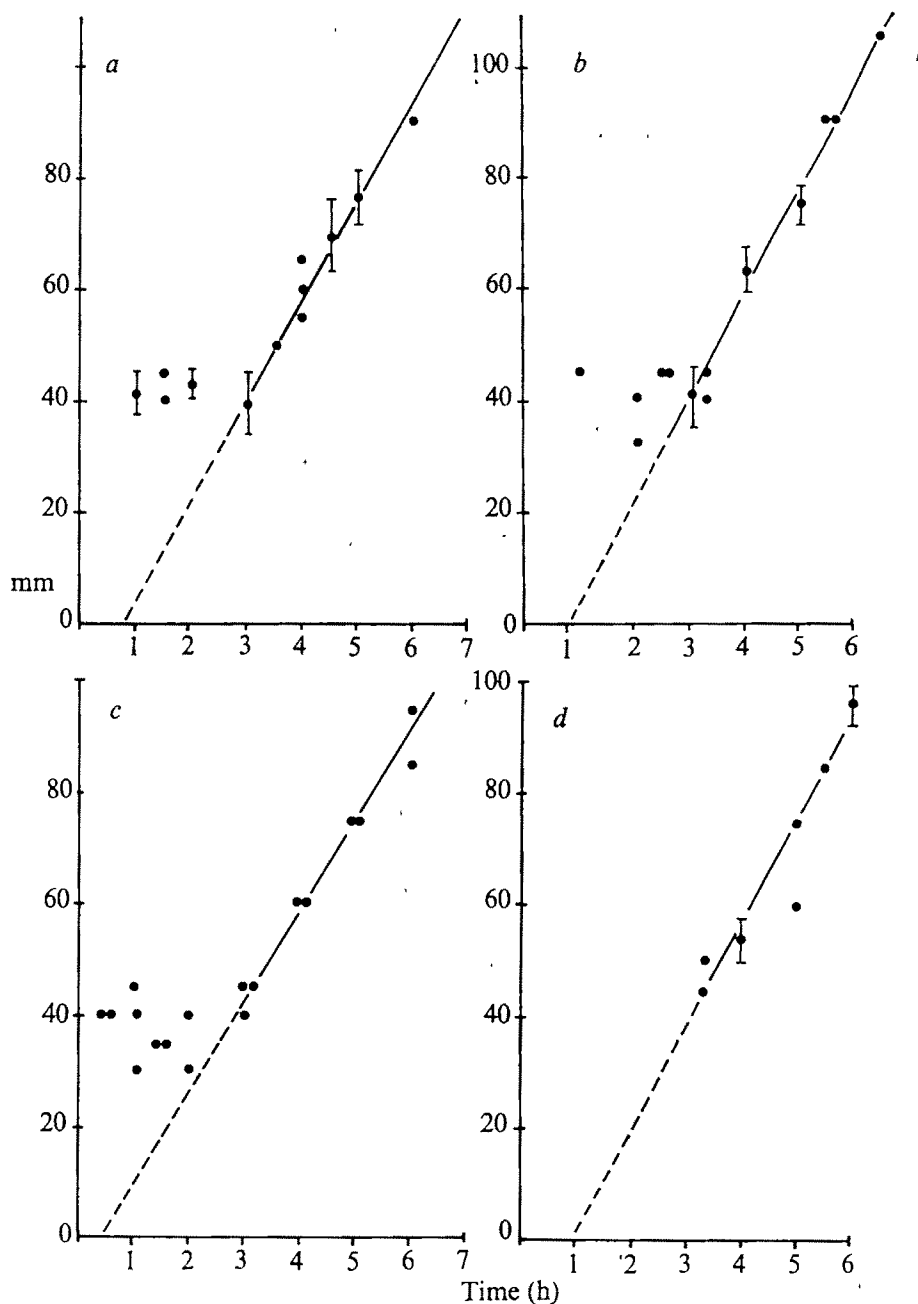


Fig. 1 Velocity of axonal transport. Ordinates, distance of wavefront of activity from most proximal site of injection; abscissae, time elapsed since injection. Where 4 or more points were obtained for any time, the means \pm s.e.m. have been plotted. Regression lines were fitted by the least squares method and were extrapolated to the time axis. Note that at intervals of less than 3 h the wavefront remained stationary at approximately 40 mm from the site of injection. Velocities of transport calculated from the slope of the regression line were: a, controls, 431 ± 16 mm d⁻¹ (mean \pm s.e.m.); b, pargyline (75 mg kg⁻¹), 458 ± 26 ; c, pargyline (25 mg kg⁻¹), 391 ± 69 ; d, reserpine (2.5 mg kg⁻¹) 448 ± 35 .

In a group of rats treated with pargyline (25 mg kg⁻¹) the sciatic nerve was crushed at the time of precursor injection, and the time when transported protein began to accumulate proximal to the crush was measured. The velocity of transport, calculated from the onset of accumulation, was $412\text{--}436$ mm d⁻¹.

At distances of less than 40 mm from the site of injection, the velocity of axonal transport could not be measured reliably owing to the presence in the

ventral roots of high ³H activity (Fig. 1). This activity appeared within 30 min of the precursor injection. Three hours after injection the 'mobile' wavefront broke away from this 'stationary' wavefront and proceeded along the axon. In the region of the stationary wavefront, 50% of the total ³H activity was TCA soluble and presumably represented the diffusion of free ³H-leucine from the site of injection. Beyond the 40-mm limit, which coincided with the exit of the sciatic nerve roots from the

spinal canal, the TCA-soluble activity fell to 10%. Boegman *et al.*² measured total ³H activity and obtained wavefronts located 40 mm from the injection site: there is no indication that they followed wavefronts into more distant regions of the sciatic nerve in order to obtain regression lines for determination of velocity. I suggest that the apparent increase in velocity that they report results from measurements made on a stationary wavefront of diffused activity at shorter time intervals for pargyline-treated than for normal animals.

It therefore seems that changes in amine levels produced by pargyline and reserpine have no effect on the velocity of axonal transport of protein. A further study³ of axonal transport involving manipulation of amine levels requires re-evaluation in the light of these results.

The work in my laboratory is supported by the Medical Research Council of Canada. I am grateful to Ms G. Bilborough and Ms B. Haft for assistance and to Mr L. Baue for the fluorimetric assays. Pargyline (Parsidol) was provided by Abbott Pharmaceuticals.

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¹ Yu, M. U., Wright, T. L., Dettbarn, W. D., and Olson, W. U. *Neurology*, 24, 237-244 (1974).

² Boegman, R. J., Wood, P. L., and Pinaud, L. *Nature*, 253, 51-52 (1975).

³ Bisby, M. A. *Expl Neurol*, 50, 628-640 (1976).

⁴ Steg, G., *Acta Physiol. scand.*, 61, suppl. 225 (1964).

⁵ Boegman, R. J., and Wood, P. L., *J. Neurochem.*, 26, 737-740 (1976).

BOEGMAN *et al.* REPLY—We agree with Bisby¹ that pargyline does not alter the bulk flow of material down the rat sciatic nerve. In our published data² we reported on the total radioactivity present in nerve segments and not the TCA-precipitable activity. This led us to conclude that pargyline alters the rate of fast axoplasmic flow.

We disagree with Bisby's comment¹ that axonal flow rates cannot be measured within the first 40 mm since we have shown that puromycin will abolish a front of TCA-precipitable radioactive material within this distance.

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¹ Bisby, M. A., *Nature*, 263, 382-383 (1976).

² Boegman, R. J., Wood, P. L., and Pinaud, L., *Nature*, 253, 51-52 (1975).

Large scale extinctions

THOMSON'S contribution¹ to the search for the cause of major extinctions points out some patterns of temporal

diversity change that merit consideration. I wish to note an important inconsistency in Thomson's argument and to discuss some work which contradicts his hypothesis of extinction through specialisation.

Thomson mentions that the diversification portion of his diversity curves are typically exponential, not logistic in shape. Later, however, he describes this diversification as "species packing" and consequent niche reduction". Such species packing should result in a logistic growth of diversity², not the exponential one that he notes. Cisne³ demonstrated such a logistic increase in the morphological specialisation of Phanerozoic aquatic free-living arthropods and also showed that diversification accompanies this specialisation, as suggested by Thomson. The apparently broad time intervals used by Thomson may prevent the detection of a logistic pattern of diversification.

That specialised taxa tend to become extinct is one of the most plausible assumptions of evolutionary biology and forms the basis of Thomson's explanation. The only test of the extinction through specialisation hypothesis failed, however, to detect any significant correlation between specialisation and evolutionary longevity within aquatic free-living arthropods³. Furthermore, Thomson's suggestion that diversification (and specialisation) is "entrained" may inadvertently revitalise the corpse of orthogenesis.

Thomson has pointed out some phenomena of major evolutionary significance—the apparent symmetry of the diversity curves and the existence of "group diversity curves"^{4,5}. I agree that it is important to focus on the causes of these phenomena rather than on one striking but merely consequential aspect—extinction.

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¹ Thomson, K. S., *Nature*, 261, 578-580 (1976).

² Strong, D. R., *Proc. natn Acad Sci U.S.A.*, 71, 2766-2769 (1974).

³ Cisne, J. L., *Evolution*, 22, 337-366 (1975).

⁴ Flessa, K. W., Powers, K. V., and Cisne, J. L., *Paleobiology*, 1, 71-81 (1975).

⁵ Newell, N. D., *J. Paleontol.*, 26, 371-385 (1952).

⁶ Flessa, K. W., and Imbrie, J., in *Implications of Continental Drift to the Earth Sciences*, 1 (edit by Tarling, D. H., and Runcorn, S. K.), 247-285 (Academic, London and New York, 1973).

THOMSON REPLIES—Flessa¹ has read into my highly qualified statements more than I actually said. I merely stated that the diversity curves "approached" an exponential pattern. I tried to explain the curves only by "analogy" with "species packing" and

in any case was working at the genetic level. I do not believe that the patterns of diversification that I described have anything to do with orthogenesis. The question of how one would recognise in fossils the specialisations that could result in extinctions is a very difficult one. Not all specialisations will necessarily be associated with extinction and the reverse is also true. If analogy with any process like species packing can be upheld, it will probably turn out that the crucial specialisations were ones of behaviour and ecology rather than of major external morphology. In that case one might expect morphological specialisation to correlate better with diversification than extinction.

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The creeping-film phenomenon of potassium chloride solution

BIN-JUINE HUANG and JEN-CHI HUANG¹ state that the creeping-film phenomenon of potassium chloride solutions cannot be satisfactorily interpreted "based on present knowledge of either surface chemistry or transport theory." We believe the phenomenon can be explained by using ordinary physico-chemical surface forces such as capillary action in a type of 'wick' effect.

Consider a glass beaker containing a saturated solution of potassium chloride; when some of the solution evaporates, crystals form on the sides of the beaker at the surface of the solution. The solution is then drawn up into micro-fissures and crevices in the crystals by capillary action, and when the solution reaches the edge of the crystal formation it wets the glass surface, and on further evaporation forms more crystals which advances the crystal front causing the creeping phenomenon observed. This is analogous to a 'wick' effect where the KCl crystals act as a wick for the solution. This would explain the creeping which occurs over the brim of the beaker.

A saturated KCl solution will exhibit creeping effects when it wets a substrate surface, and this has been observed in a variety of substances, such as glass, aluminium and lucite plastic (A. M. Yacynych, unpublished). It would be difficult to explain this phenomenon by invoking a specific interaction between the solution ions and the substrate surface, in view of the diverse chemical nature of the substances on which this phenomenon occurs. This creeping effect does not occur when the KCl solution does not wet a substrate surface, as is the case with Teflon (A. M. Yacynych, unpub-

lished). Also, creeping does not occur if the KCl solution is unsaturated, or if a beaker containing a saturated solution is stoppered. In the case of an unsaturated solution, if any crystals were formed on the sides of the beaker they would be dissolved as the unsaturated solution was drawn into any fissures or crevices in the crystal and the creeping could not occur. In the case of a covered beaker, no creeping occurs because the atmosphere inside the beaker is saturated with water vapour, preventing evaporation of the solution which is necessary for the crystal front to form and advance.

Regarding the creeping which occurs through a 'seamless' glass tube connector; the authors do not specifically mention whether the joint was greased or ungreased. In the former case, we would not expect any creeping to occur, however, in the later case, small fissures and crevices are certain to be present in the joint allowing the creeping to occur. In fact, this ability of the KCl solution to creep into small fissures is put to good use by electrochemists in constructing reference electrodes². Instead of using a porous frit or a salt bridge to maintain solution contact, a platinum wire is sealed in glass and contact is maintained by way of the small fissures which form between the platinum and glass on cooling.

The alkali halides form two separate isomorphous groups³: the lithium and sodium salts; and the potassium, rubidium, and caesium salts. If the creeping results simply from the wick effect by the crystals, one would not expect it to be unique, and in fact, compounds such as potassium bromide, potassium iodide, and caesium chloride that are crystallographically isomorphous with KCl also exhibit this creeping phenomenon (A. M. Yacynych, unpublished). Sodium fluoride, chloride, bromide and iodide which are not isomorphous with KCl do not creep. Perhaps their crystal formation does not have the small fissures and crevices necessary for the wick action.

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CONCERNING the paper by Huang and Huang¹, may I suggest that the well known Marangoni Effect may provide the authors with an explanation for their observations. A good account of different types of Marangoni Effect may be found in ref. 2.

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¹ Huang, B. J., and Huang, J. C., *Nature*, 261, 36-38 (1976)

² Scriven, L. E., and Sternling, C. V., *Nature*, 187, 186-188 (1960)

Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

HUANG and Huang¹ describe the flow of potassium chloride solutions over surfaces and conclude by saying the results are "inexplicable" but that they might be attributed to "interactions of the ions with the surface of the beaker or tube and the surrounding gaseous molecules". In 1950 I observed the same effect in aqueous solutions of potassium ferricyanide, but results of several experiments were not published because I was unable to obtain reproducible quantitative results. I attributed the phenomenon to surface mobility of ions and molecules.

Evidence for surface mobility of adsorbed atoms was first given by Volmer and Estermann in 1921. They set up an experiment in which mercury evaporated from a bath at -10°C to a glass plate at -65°C . Hexagonal crystal plates of mercury formed on the glass. The crystals should have grown at the rate G/p in which G is the rate at which mercury molecules struck unit area of crystal at the glass surface and p is the density of the crystal. But the dimensions of the crystals grew $1,000\times$ faster than that. Volmer and Estermann concluded the

mercury atoms moved over the surface of the glass to become attached to the crystals.

A few years later, Volmer and Adhikari placed a benzophenone crystal between 0.02 to 0.09 mm from the edge of a glass plate; a mercury drop just touched the edge of the glass. The crystal dissolved in the mercury due to the mobility of benzophenone molecules over the surface of glass (vapour pressure negligible); it was estimated that the resistance to diffusion over the glass was one hundredth of that in the liquid state.

Wicke in 1940 was the first to suggest that molecules adsorbed from the vapour phase contribute to total flow through porous materials; and the following year Wicke and Kallenbach flowed mixtures of CO_2 and N_2 past one face of a charcoal membrane, at 600 torr total pressure and pure N_2 past the other face at the same pressure. In the absence of a pressure gradient there was no flow through the membrane but diffusion of CO_2 into N_2 could occur and the diffusion coefficient could be determined by analysis. Experiments were done in the range $0-300^{\circ}\text{C}$. From the laws of gas kinetics, the diffusion should increase with temperature and this was observed at high temperatures. At low temperatures, however, the diffusion coefficient increased as temperature was decreased, which was attributed to the greater adsorption of CO_2 at lower temperatures. Mobility in the adsorbed phase contributed as much as 50% of the total flow of CO_2 at the lowest temperature.

So, when crystals are in contact with another solid surface an adsorbed layer of atoms (or molecules) can form on the solid surface in dynamic (mobile) equilibrium with the crystal. When a vapour (or gas) is in equilibrium with a solid surface there will be an adsorbed phase on the solid in kinetic equilibrium with the vapour; atoms or molecules will be mobile over the surface within the adsorbed phase. Evidence for this is considerable²⁻⁴. Adsorption also occurs when liquid mixtures and solutions are in contact with solid surfaces and various forms of adsorption isotherm have been found experimentally, see for example, Kipling⁵. Atoms and molecules will be mobile within that adsorbed layer.

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¹ Huang, B. J., and Huang, J. C., *Nature*, 261, 36-38 (1976)

² Heineman, W. R., and Kuwana, T., *Analyt. Chem.*, 44, 1972-1978 (1972)

³ Mellor, J. W., *A Comprehensive Treatise on Inorganic and Theoretical Chemistry*, II, 531-532 (Longmans, Green and Co., New York, 1922).

¹ Huang, B., and Huang, J., *Nature*, 261, 36 (1976).

² Brunauer, S., *The Adsorption of Gases and Vapours* (Oxford University Press, 1944)

³ Carman, P. C., *Flow of Gases and Vapours through Porous Media* (Butterworths, London, 1956)

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⁵ Kipling, J. J., *Adsorption from Solutions of Nonelectrolytes* (Academic, London and New York, 1965).

reviews

Golden jubilee monograph

J. H. S. Blaxter

The Eggs and Planktonic Stages of British Marine Fishes By Sir Frederick S. Russell Pp. xv+524. (Academic; London, New York, San Francisco, 1976.) £19.50.

THERE is a tendency, as fisheries come under increasing pressure, for the success of the fishery to become dependent on the recruit fish rather than on a longer run of successful year-classes. The study of the pre-recruit phases of the life history, both for the purpose of prediction as well as for understanding what determines success or failure of a year-class, then assumes greater importance. This is one of the reasons why investigations of eggs, larvae and juveniles of commercial fish species have been greatly stepped up over the past twenty years. Both sea surveys of these young stages, as well as laboratory experiments involving rearing from the egg, are now part of the standard repertoire of most larger fisheries laboratories, and it is now generally accepted that the year-class strength is probably determined in the first year of life.

It is surprising that no standard work of identification of the young stages of north-eastern Atlantic species has been written since Ehrenbaum's *Eier und Larven von Fischen* which formed the first volume of his *Nordisches Plankton*, produced between the years 1905 and 1909. Many imperfections in identification existed at that time. To a limited extent they were made good in subsequent publications on restricted groups of species such as the gadoids by Johannes Schmidt in 1909, or in more comprehensive descriptions for other areas, as in the Fauna and Flora of the Gulf of Naples during the 1930s and in the ICES plankton identification sheets that appeared after World War II.

Sir Frederick Russell's book thus fulfills a clear need. It is a first class and completely fresh re-appraisal of the identification problem. It covers the planktonic stages of coastal species of northern European waters likely to be found within the 200-m depth contour but it excludes strictly oceanic species. Introductory chapters on terminology, development, principles of identification, feeding habits

and vertical and horizontal distribution are followed by the main section where each teleost family is taken in turn. A description, with key where appropriate, is given for the egg, larva and post-larval stage. Relevant ecological or experimental work is also referred to—one aspect of the book which makes it quite different from previous publications.

The value of the monograph lies therefore in the collation of the taxonomic and other publications, and the assembling and up-dating of the many keys scattered in the literature. During this process the author has brought to bear his unique experience in this field, to improve, clarify and amplify the keys.

The reference to many 1975 and even some 1976 scientific papers in the bibliography is evidence of his success in making the monograph as comprehensive and up-to-date as possible within the range of species adopted. It

is, however, a pity that the style and content of the book, limited as it is to northern European fishes, mean that some of the excellent and relevant ecological and experimental work done, for example, on the Californian sardine and anchovy, has perforce had to be excluded.

Sir Frederick Russell retired from the directorship of the Plymouth laboratory in 1969 and this monograph is a *tour de force* representing the results of his enviable energy during subsequent years. He published his first paper on fish eggs and larvae in 1926 so that the monograph is a fitting and admirable golden jubilee offering to the scientific community. We all look forward to the celebration of his diamond jubilee! □

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Energetic ion beams and sputtering

Ion Implantation, Sputtering and Their Applications. By P. D. Townsend, J. C. Kelly and N. E. W. Hartley Pp. ix+333. (Academic: London and New York, June 1976) £13; \$32.25.

IN a great many practical situations the behaviour of a component depends on the near-surface composition, physical state or topography of the material. Examples spring to mind in the optical, mechanical, electrical and corrosion fields, and the extraordinary developments in semiconductor devices and circuits involve a sophisticated control of material properties within a layer a few microns thick.

Diffusion, coating and etching have hitherto been used to control surface composition and its lateral variation, but these are no longer precise, versatile or reproducible enough for some requirements. The past decade has seen the development of an entirely different set of techniques based on the use of energetic ion beams by means of which chosen atomic species can be injected into a surface, or, by sputtering, material can be removed.

This book provides a very timely review of the progress achieved so far

in a field in which industrial applications are being extended as each month goes by. The fundamentals of the subject are treated admirably, from the slowing-down of the incident ions to their effects on the target structure. The treatment of applications is more superficial, but the authors must be conscious of the speed with which a detailed account would become outdated. Readers, and there will be many, who are mainly interested in the ion implantation of semiconductors will be disappointed: the literature of this is now so extensive that the authors, perhaps wisely, have chosen to concentrate on the remainder.

A bonus, for which the reader is not well prepared by the title of the book, is a lengthy and most useful chapter dealing with the analytical applications of ion beams.

I congratulate the authors on their clear and readable treatment, and the publisher for the attractive presentation of text and diagrams.

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Describing the brain

Cell Biology of the Brain. By W. E. Watson. Pp. xi+527. (Chapman and Hall London, August 1976.) (Distributed in the USA by Halsted: New York.) £15.

THERE have been previous attempts to describe the brain in the language of cell biology, but few have been as crammed with information or as well written as the present monograph. The author has not been intimidated by this heroic task, and has produced an eminently readable and useful volume. The first six chapters give a broad outline of the cellular properties of the brain, its origin from epithelial tissue, the differentiated properties of neurones, current understanding of membranes and their function, and a fairly extensive review of modern knowledge of the 'chemical transducer' mechanisms by which neurones respond to chemical signals.

This includes a description of recent biochemical techniques for the study of receptor sites by the ligand binding approach, the coupling of receptors to cyclic nucleotide mechanisms and more general biochemical changes in neurones associated with activity. In this section there is little emphasis on the neurone as a carrier of electrical signals, nor is there any description of the presynaptic machinery associated with the production, storage and release of neurotransmitters—a deliberate omission since the author rightly points out that these topics are already dealt with extensively elsewhere. But this perhaps leaves some major gaps in coverage for student readers.

The largest chapters, and the most valuable in my estimation, were the remaining four, which deal with the topics of plasticity in the nervous system, responses to injury, neurotrophism and genetics. These are topics which encompass some of the most fundamental problems of neurobiology, and the author is an expert in this area. He gives an excellent review, taking examples from a broad spectrum of vertebrate and invertebrate studies. In dealing with this large area the author deliberately, and probably wisely, avoids the even larger topic of developmental neurobiology, but his deliberate omission of any discussion of the nerve growth factor seemed to me a curious decision.

The book is undoubtedly well referenced. Indeed some might consider that the 3,247 citations in the bibliography (which occupies almost 200 of the 527 pages) were enough for six

volumes of this size. I would personally have preferred to have seen fewer references and more illustrations. Overall, however, there is no doubt that the book is an unusual and valuable source of information on topics that are not well reviewed elsewhere.

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Neuromuscular junction

Neuromuscular Junction. (Handbook of Experimental Pharmacology. New Series, Vol. 42.) Edited by Eleanor Zaimis. Pp. xvii+746. (Springer Berlin and New York, 1976) DM 280; \$91.90.

THIS latest volume in this well-known series of reviews and monographs, which all pharmacologists turn to for information when everything else fails, lives fully up to the standards of its predecessors. Each of its chapters amounts to a substantial review article and like others in the series, the book as a whole is full of detail, with each chapter accompanied by an exceptionally complete and valuable bibliography.

The neuromuscular junction is, without any doubt, the most intensively studied synapse of all, it is also a highly atypical synapse, being a simple one-to-one connection between a nerve fibre and a muscle fibre. In life it serves simply as a relay (and a highly reliable relay at that, very rarely affected by disease) and has none of the integrative properties that characterise most other synaptic connections. To some investigators studies of the neuromuscular junction are justified mainly by the implications that the results hold for other more complex synapses. Not to all, though, for at a recent scientific meeting an eminent investigator, asked whether the neuromuscular junction helped to explain brain function, replied that all he could be sure of was that the brain was indispensable in understanding the neuromuscular junction.

This book certainly does not lend much encouragement to the view that the neuromuscular junction is a good model for other synapses. The admirable article by MacIntosh and Collier on the metabolism of acetylcholine makes a point of comparing the results of work on the neuromuscular junction, where electrophysiological

techniques have generally proved much more valuable than biochemical techniques, with work on the brain, where the reverse is true. The differences between the two are a good deal more obvious than the similarities, and the authors comment gloomily: "The extent of these peculiarities of individual tissues... may seem depressing to investigators who would like to think that their results have a general significance and should help to explain all analogous phenomena". Particularly valuable in this article is the balanced discussion of the present status of the vesicle hypothesis. The evidence, which is complicated and often conflicting, is presented with an economy and a clarity which is all too rare in scientific writing.

The following chapter, by Ginsborg and Jenkinson, equally well-written, surveys the mechanism of impulse transmission mainly from an electrophysiological point of view; it, too, succeeds in distilling an immense amount of information into a concise and coherent account. What emerges most clearly from these two chapters is the considerable uncertainty that still exists about the mechanism of acetylcholine release and the difficulty in relating the results of electrophysiological experiments to biochemical events within the nerve terminal. These two chapters, together with the preceding one on morphology by Bowden and Duchon, form the first half of the book and complement each other extremely well.

The rest of the book is concerned mainly with neuromuscular blocking drugs and anticholinesterases. The contentious areas here are the mechanisms of action of depolarising blocking agents and of anticholinesterases, well-scarred battlegrounds on which relatively little new evidence has appeared in recent years. I would have preferred to see less discussion of these topics and fuller accounts of the recent biochemical advances in isolating and characterising acetylcholine receptors. Studies based on these advances have already led to a reappraisal of the nature of the defect in myasthenia gravis and show great promise of leading to a much better understanding of the process underlying synaptogenesis and denervation effects; yet they receive only fleeting comment. The book will, however, be invaluable as a work of reference. The only danger is that it may attract even more experimenters to assault this unoffending synapse.

H. P. Rang

H. P. Rang is Professor of Pharmacology at St George's Hospital Medical School, London, UK.

New Books in the Neurosciences

Techniques and Basic Experiments for the Study of Brain and Behavior

by J. BUREŠ, O. BUREŠOVÁ and J. P. HUSTON.

1976 xvi + 272 pages. US \$23.25/
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Membrane Morphology of the Vertebrate Nervous System

A Study with Freeze-etch Technique

by C. SANDRI, J. M. VAN BUREN and K. AKERT.

PROGRESS IN BRAIN RESEARCH,
Vol. 46

1976 xii + 388 pages. US \$67.50/
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Proceedings of the International Narcotics Research Club Meeting, Aberdeen, United Kingdom, 19-22 July, 1976

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Hypertension and Brain Mechanisms

edited by W. DE JONG, A. P. PROVOOST and A. P. SHAPIRO.

PROGRESS IN BRAIN RESEARCH,
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APPLIED ELECTRONICS FOR VETERINARY MEDICINE AND ANIMAL PHYSIOLOGY edited by W. R. Klemm, *Texas A & M Univ., College Station. (11 Contributors)* This book presents not only specific electronic techniques, but also briefly explains many electronic fundamentals. The contents are organized around certain topics relevant to the diagnosis of animal diseases and animal research. Included are basic bioelectricity, fundamentals of electronics, electronic measurement of flows, pressures and strains, electrocardiography, electromyography, electronic function of motor function and dysfunction, electroencephalography and echoencephalography. A section on the safe use of electronic equipment discusses the dangers of electric shock, the operation and care of electronic equipment, safety standards, and practical safety guidelines. '76, 484 pp., 236 il., 27 tables, \$31.00

ESSENTIALS OF BACTERIAL AND VIRAL GENETICS by David M. Carlberg, *California State Univ., Long Beach.* Beginning with an introductory chapter on the fundamentals of modern genetics, this book goes on to discuss the chemical and biological nature of molecules responsible for the storage and transfer of hereditary information, the genetic code, polypeptide synthesis and its control, molecular biology of spontaneous and induced mutations, and characteristics and isolation of bacterial mutants. A general discussion of recombination is followed by coverage of transformation and conjugation in bacteria. Genetics of viruses, eucaryotic microorganisms, and the higher plants and animals are also included. '76, 336 pp., 166 il., 35 tables, \$24.75

ENVIRONMENTAL PROBLEMS IN MEDICINE edited by William D. McKee, *Palo Alto Medical Clinic, Palo Alto, California. (36 Contributors)* Understanding the importance of increasing environmental hazards and their contribution to the development of disease is the goal of this book. It covers the population problem, changes in living patterns in modern society, and effects of various hazards and pollutants in our environment. Health effects related to food, water and air in modern societies are discussed, as well as the role of chemical carcinogens, mutagens and teratogens. Specific pollutants such as ionizing and nonionizing radiation, agricultural chemicals and pesticides, and environmental metals are discussed in separate chapters. Special emphasis is placed on the major diseases causing death in our modern society. '75, 880 pp. (6 3/4 x 9 3/4), 90 il., 152 tables, \$36.50

ANIMAL DISEASE MONITORING edited by D. G. Ingram, W. R. Mitchell and S. W. Martin, *all of the Univ. of Guelph, Guelph, Ontario, Canada. (25 Contributors)* The current state of knowledge and practice of animal disease information systems is discussed in this volume. Also presented are mathematical and computer-based epidemiological models of animal disease which will provide much useful information for teaching and policy decisions in animal disease control programs. This volume will also be valuable for providing significant information to optimize animal productivity, minimize losses caused by disease, and guard public health. '75, 228 pp., 40 il., 36 tables, \$21.50

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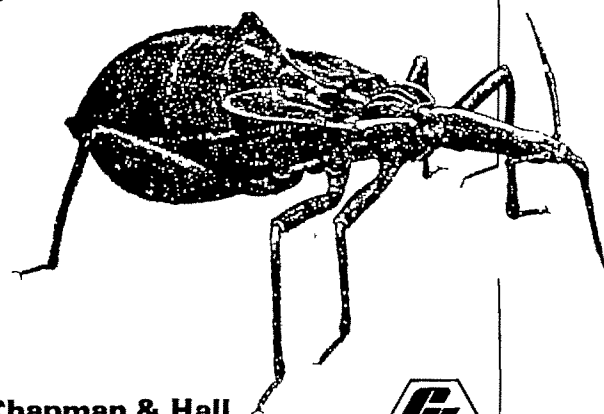
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Extragalactic astronomy

Galaxies and the Universe. (Stars and Stellar Systems: Compendium of Astronomy and Astrophysics, Vol. 9) Edited by A. Sandage, M. Sandage and Jerome Kristian. Pp. xxii+818. (University of Chicago: Chicago and London, 1976.) £30; \$45.00.

It is 50 years since Edwin Hubble discovered Cepheid variable stars in the M31 galaxy, an epochal step in surveying the extragalactic universe, 21 years since this series was conceived, and 14 years since Allan Sandage commenced the editorial work for this book. The result is a book which is a product of the 1960s and which contains articles completed between 1965 and 1973, with a mean epoch of 1970. Is a book six years out-of-date worth £30?

Fortunately that is an improper question: the 19 chapters are written by people with the highest reputation, each contributing in a field in which he or she is an expert. As Sandage rightly says in his preface, one remarkable feature is the enduring quality of the chapters, which are definitely not out-of-date, although some (for example, quasars, clusters, radio emission) are certainly not up-to-date. The editorial brief was to provide a compendium of essential facts about the Universe, and this policy has ensured that ephemeral theoretical playthings and tentative observations of implausibilities have been kept to the irreducible minimum. Not an easy task in extragalactic astronomy!

Galaxies and the Universe provides a review of well-established knowledge on the observed properties of galaxies and quasars. Individual chapters discuss the contents, masses, magnitudes, colours, sizes, energy distributions, and radio properties of galaxies and quasars. There are also extensive contributions on the dynamics and structure of galaxies. Observational cosmology is dealt with in sections on the distance scale of the universe, the distribution of galaxies, and an impressive review by the editor entitled "The Redshift".

The style is that of a review addressed to an informed reader, and there are very extensive bibliographies: Over 1,000 astronomers feature in the Name Index, and 1,200 objects in the Galaxy Index: these statistics give an impression of the ambitious scope of the work.

Galaxies and the Universe stands as a fitting synoptic monument to a heroic age in the quest to explore the Universe. Here are the results of a golden half-century that successively established the existence of island galaxies

beyond the Milky Way, the redshift, radio galaxies, the cosmic background radiation, and quasi-stellar objects. The nature and history of the Universe on the largest scales is now yielding to the methods of science, and the main thrust of astronomical research for the rest of this century will be among the galaxies. Here in broad outline is a competent summary of what we know about those remote objects, and an indication of what we need to find out. It is an absolutely invaluable work of reference for anyone working in the field and an essential purchase for libraries. It is probably the most significant astronomical book published this year.

Simon Mitton

Simon Mitton is Secretary of the Institute of Astronomy, University of Cambridge, and Editor of the Quarterly Journal of the Royal Astronomical Society.

Amphibian physiology

Physiology of the Amphibia Vol. 3. Edited by Brian Lofts. Pp. xiv+644. (Academic: New York and London, July 1976.) \$58.50; £35.70.

BECAUSE of their phylogenetic position and varying degrees of adaptation to life in two media the Amphibia are a group with which comparative physiologists are rightly very much concerned. In this, the presumably final, volume of *Physiology of the Amphibia*, the somewhat scattered literature in several fields is brought together and critically reviewed. The completed series now constitutes a source of information which should be indispensable to all herpetologists who are physiologically inclined. The timescale for the appearance of the various parts has, however, been unduly extended and it is a measure of the advances which have been made in some aspects of the subject in the intervening twelve years that there are articles on metamorphosis in both volumes one and three. Regrettably the prices of these same two volumes are an even more striking testament to the pace of inflation.

The present volume covers integrative aspects such as colour change (J. T. Bagnara), moulting (L. L. Larsen) and resistance to desiccation (E. Elkan); cell culture (K. A. Rafferty) and immunity mechanisms (E. L. Cooper); general neuroanatomy (A. Oksche and M. Ueck); more specialised articles on the visual (D. Ingle) and auditory (R. C. Capranica) systems; and finally metamorphosis, with special emphasis on its biological and eco-

logical aspects (M. H. I. Dodd and J. M. Dodd). The declared editorial policy has been to group related topics in the same volume but perhaps inevitably the final one seems to be the most heterogeneous, although there is more interrelation between some of the chapters than might at first appear. For instance it is helpful to have the account of the neuroendocrine systems alongside the discussion of the role of neurosecretion in metamorphosis.

The authors of the articles on cell culture and immunity mechanisms both stress the advantages of using amphibian material for general work in these fields; if this should lead to stocks of Amphibia being maintained in more laboratories, then the homily on amphibian husbandry in the chapter on pathology (E. Elkan) is very timely. At least as far as the more terrestrial species are concerned it must be realised that Amphibia in captivity are pathological specimens unless the conditions closely reproduce those found in the natural habitat.

The book is well produced and printing errors are rare, and then only of a minor nature

C. L. Smith

Dr Smith is a Lecturer in the Department of Zoology at the University of Liverpool, UK.

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Immunobiology of reproduction

The Immunobiology of Mammalian Reproduction. By A. E. Beer and R. E. Billingham. Pp. xvi+240. (Prentice-Hall: Engelwood Cliffs, New Jersey, 1976.)

In their preface to this volume, Beer and Billingham point out the absence of any previous concise, but comprehensive, account of the principles of the immunobiology of reproduction. There is of course good reason for this deficiency. A problem which requires considerable knowledge and understanding of subjects as diverse as immunology, oncology, reproductive and developmental biology, genetics and endocrinology makes discussion hazardous if the authors are not to be caught in the crossfire of experts. That this demanding combination of talent and expertise is required is testimony to the fundamental importance to biology of the problems under consideration. If we are to judge this ambitious work by the authors' professed aim to make good the existing deficiency, we can consider the volume as at least a qualified success.

Not surprisingly, the authors cover comprehensively that data on immunological aspects of foeto-maternal relations available up to mid-1975. This area, in which the authors have both made a major experimental contribution themselves and stimulated new theoretical approaches, is considered critically and with a balanced combination of clinical and experimental evidence. Wide and important issues are covered, such as the possible reasons for a highly polymorphic system of tissue antigens and the resistance to rejection of the foetus or its malignant derivatives. Although a great deal of data is presented, it is, however, often in a form not conducive to logical exploration of the problem. For example, discussion of the question of trophoblast antigenicity was dispersed to several points in the text for no obvious reason, and at times this led to an impression of repetitiveness even when new information was imparted. At the end of this series of chapters, one was left with an element of intellectual indigestion, as though the authors had not had sufficient time to complete their synthesis of the data and analysis of the problem. We certainly do not gain a clear impression of either current opinion or the authors' opinion on the mechanism by which the mammalian foetus survives *in utero*.

Elsewhere in the volume there are some important omissions for a book

on immunoreproduction—for example, immunity to the hormones of reproduction and to ovarian tissue are not considered at all, and immunity to products of the male genital tract are discussed rather briefly. It is also evident, understandably, that the authors are ill at ease in discussing some aspects of the subject. For example, in the chapter on reproductive biology, the impression is given that uteri of all species secrete blastokinin, that the function of this protein is agreed and that 'gastrulation' occurs in the oviduct.

In spite of its deficiencies this book will be a useful source of reference for those workers actively engaged in the investigation of this problem. For others with a more peripheral interest in the subject of immunoreproduction, the volume may prove rather hard work, but will acquaint them with the varied, rather confused, but immensely exciting problems of such central importance to biology.

Martin H. Johnson

Dr Johnson is a Lecturer in Anatomy at the University of Cambridge, UK.

Conversations in culture

Neurophysiologic Studies in Tissue Culture. By Stanley M. Crain. Pp. xii+280 (Raven: New York, 1976.) \$25.50.

It is indeed a pleasure to rely on the insight of someone with almost 15 years of experience 'listening' to the conversations which nerve cells carry on in culture. Crain has put together a strong concoction of his own data as well as several others' in the field. He has added spice through description of his role as a kind of ambulance driver to transport the live cultures through hectic New York City traffic.

The monograph begins with an orientation which relates his approach to that of others in the study of brain electrical function, and includes a brief historical note on the progress through which recording from cultured cells has grown. The second chapter deals briefly with methods of growth of cells and techniques for recording action potentials with either extracellular or intracellular electrodes. The author then details the synaptic potentials which have been obtained from dorsal root and sympathetic ganglia.

The major portion of the book is then concerned with recording from explants of central nervous system tissues. In each case the recordings amply demonstrate both the prolonged slow wave effects of stimuli, the oscillatory after-discharges and the excitatory and

inhibitory post-synaptic potentials. The electrical tracings are well salted with both light and electron microscopic correlates.

The technique of dissociating brain into single cells and then studying their electrical activities in culture is only a few years old, relative to explants, but the dissociated cells have revealed that their behaviour is quite similar to that of the traditional explants. Once again, Crain discusses the peripheral nervous system as distinct from the central nervous system.

The next chapter deals with responses to various pharmacological agents, including curare, strychnine and bicuculline, as antagonists to the major transmitters, acetylcholine, glycine and GABA, respectively. There is a useful discussion of the results of serum from patients with multiple sclerosis and animals with experimental allergic encephalomyelitis. These sera have complex effects on both neurones and myelin. He then deals with the possible relationship of spontaneous fluctuations in electrical activity to the electroencephalogram. This is followed by a discussion of the important question of longer term 'trophic' interactions of neurones and the cells with which they synapse. Unfortunately, the consideration of the important 'supportive' role of glia is examined in an earlier chapter; one is therefore obliged to shuffle back to obtain the best synthesis. However, Crain certainly appreciates the relevant functional as well as developmental significance of the 'neuro-glial' unit. These findings also touch the wider context of nerve-myelin trophism, as in the classical studies of M. Abercrombie and J. Z. Young.

The final chapter, and other sections throughout the book, contain the lessons of many years: beware simple morphological analysis, the need for standardised ingredients in cultures, attention to the state of the media as related to its last change and the importance of cell-cell contacts (that is, cell number). He ends on a positive note when confronted with the exceptional rise in both the number of interested workers and the collateral spin-off to other disciplines. The only obvious 'omission' is the study of the nerve cell lines, but as Crain certainly appreciates, this could be a monograph in itself. This is a masterful synthesis of not only his own extensive experience, but also the best of others, by an acknowledged founder-father of the art.

E. J. Thompson

Dr Thompson is Senior Lecturer in Neurochemistry at the Institute of Neurology, and Honorary Consultant to the National Hospital for Nervous Diseases, London, UK.

nature

December 2, 1976

Swords to ploughshares: is it possible?

A NEW initiative is being launched today (December 2) by a group of natural and social scientists to shed some light on public attitudes to disarmament. It originated with M Albert de Smaele, a former Belgian Minister and leading figure in the Belgian Socialist Party, and has been co-sponsored in a personal capacity by a group including Professor V S Emelyanov of the Commission on Disarmament Problems of the USSR Soviet Academy of Sciences and Professor Eric Burhop, President of the World Federation of Scientific Workers.

The group rightly notes that it is difficult to arouse public opinion on security questions because of a 'mistrust of opposing theses'. So it tries instead to frame questions on armament and disarmament rather than peddle answers, and it hopes that by getting these questions to politicians, leaders of industry, trade unions, the professions, the universities, churches and so on it might help to get going again a debate which has noticeably run aground in recent years.

The premise of the questionnaire is that either the expenditure of 60% of all research and development funds and the employment of 50 million people in armed forces is futile and a senseless waste, or that sooner or later there will be the 'ultimate catastrophe'. So, run the (somewhat paraphrased) questions:

- can war solve the tensions dividing political systems?
- with the current balance of power, is security better served by competitive escalation of armaments or by balanced reduction—and might some nations in Europe lead the way in a reduction as an example?
- what course is being taken by political thought? Would a debate in Parliament, or in the Interparliamentary Union be appropriate?
- would you favour a collective, controlled commitment to forbid new scientific projects related to mass destruction accompanied by an increase in socially beneficial research; the first consequence of which would be a ban on underground nuclear testing?

● could a military-to-social transition, retaining full employment, work?

● should there be more denuclearised zones, and what further measures might help halt the arms race?

It would be easy to dismiss the questionnaire as naive, if not loaded. The first two questions are really statements—very worthy ones, of course, but calculated to encourage one to dismiss the whole thing as a leftist propaganda exercise and to read no further. This would be a pity because it really would be valuable to hear a wider range of views than is normally advanced on the question of putting military resources, particularly in research and development, to work in new fields.

The huge stumbling block, it is always claimed, is adequate monitoring. There are thirty years of distrust to be overcome—mainly worry by Western nations that the Soviet Union by the nature of its society and its geography, would be able to continue military development clandestinely. Present signs are a little more optimistic: there has recently been revived talk in the Soviet Union about permitting on-site inspection in nuclear test monitoring. Even so, the sponsors of the questionnaire should leave Moscow in no doubt of the disquiet that monitoring questions are bound to raise when it is research and not hardware that is being observed.

It would, nevertheless, be good to get some intelligent non-doctrinaire assessment of what potential there is within the defence industry for a change in purpose. And the first step in that direction is to see whether there are any good ideas around for how a task so financially, politically and technologically complicated could be fulfilled. If the questionnaire did no more than start a modest debate on changing objectives for the 1980s it might have started something worthwhile. For too long military R&D has been regarded as too sacred to touch. □

Fear of flying

Which country gave away the largest percentage of its GNP as foreign aid in 1974?

Which country has legislated that private manufacturing companies offer 49% of their share to employees?

Which airline flies the longest non-stop scheduled flight?

So runs a recent advertisement for Iran Air, which seems also to have a bit of a public relations job to do for Iran. The country is growing fast, thanks to its oil revenues. Its expenditure on arms quadrupled between 1972 and 1975. And because of its military spending and its oil money, it enjoys good international relations with a wide range of nations. Amongst its ambitions is clearly the development of universities and science and technology faculties the equal of those in the West. But amidst all this bustle, some detect a faint smell of the violations of human rights.

Amnesty International, in a briefing which academics might wish to read before visiting the country (40p from 55 Theobald's Road, London WC1), has tried to put together what is known about political prisoners in Iran. Even their numbers are uncertain, ranging from the three thousand that the Shah himself concedes to the tens of thousands claimed by some foreign journalists and exiles. Torture is admitted to by the Shah, and is clearly common, although evidence is not as forthcoming as in some other countries, probably because of the immense power of SAVAK, the security organisation, and the fear it instils. Justice is dispensed with little regard for human rights to those accused of political crimes in Iran. Execution for such crimes is not uncommon. Is it impossible for a rapidly-evolving newly-rich country to survive without such repression? □

Watching the rivers blow

Vera Rich looks at a USSR river diversion project which may use nuclear explosions

A RECENT resolution of the Central Committee of the Communist Party of the Soviet Union and the Council of Ministers on the need for a more rational use of water resources focuses attention once again on one of the most grandiose projects ever proposed by Soviet civil engineers—the diversion of water from the Siberian rivers flowing into the Arctic in order to augment the shrinking resources of the Caspian basin. The growing demands on Soviet agriculture after a series of disastrous harvests and the falling levels of the Caspian and Aral Seas make an injection of new water supplies into this region a prime necessity if the reclaimed virgin lands are not to return to desert and the inland seas are not to become salt-marshes. Accordingly, the final section of the resolution calls for engineering and environmental studies on the practical measures and possible ecological consequences such a plan would entail.

Massive hydroengineering projects are by no means new in Russia. Peter I (the Great) proposed to link the Volga and the Don via their tributaries, the Kamyshin and the Lavla, by a navigation canal; he was thwarted by the local governor, Prince Galitsin, who maintained that "God had made the rivers to go one way and it was presumptuous in man to think to turn them in another". Peter's daughter, the Tsaritsa Elizabeth, inaugurated a project to link her country retreat of Tsarskoe Selo to St Petersburg by canal, a scheme which was abandoned half-completed when she died in 1762. The most memorable achievement of the Tsarist era involving water concerned not irrigation but drainage—the reclamation of the Neva marshes and the construction, on this unpromising site, of the new capital of St Petersburg. Indeed, the era left no hydro-engineering works comparable with its last great engineering triumph, the Trans-Siberian Railway.

The new Soviet government soon turned its attention to the possibility of remodelling the waterways. In the 1920s, Lenin's formula of "Communism=Soviet rule plus electrification of the whole country" initiated a great drive to construct hydro-electric stations. Even during the Revolutionary war, the possibility of building a Volga-Don canal was so widely discussed that it became the

subject of black humour between Stalin and Voroshilov: such major engineering projects, it was felt, would not only be a spectacular achievement for Soviet engineering, with all the attendant publicity at home and abroad; the motif of religion which had frustrated Peter now reappeared in inverted form. The idea was that intervention to "correct a fault" of nature would be a valuable contribution to the atheist re-education campaign because the haphazard and "badly organised" geography which Soviet engineers sought to rectify clearly could not be the work of an intelligent Creator.

It is difficult to establish precisely when the idea of constructing canals for river diversion first arose. The aura surrounding the concept even now is redolent of the atmosphere of the early days of Soviet rule. By the 1950s it had become a desideratum, in one collection of essays on the future of Soviet science published in 1959, it is referred to almost casually in comparison with more modern and daring proposals like the damming of the Bering Straits or the diversion of the North Pacific currents. But the idea of river diversion, couched always in terms of the future, acquired over the years something of a millennial character comparable in a sense to the final achievement of full Communism rather than something likely to be accomplished within the next few Five-Year Plans.

Emphasising the practical

The new resolution, however, although concerned largely with preliminary work, re-emphasises the practical nature of the project and implies that during the current Five-Year Plan considerable efforts and resources will be devoted to feasibility studies and to surveys and comparison of alternative routes. The "classic" rivers proposed for such diversion are the Ob' and Enisei. According to plans outlined at the International Geographical Congress in Moscow last August a system of reservoirs, pumping stations, canals and tunnels would be built through the Tungai corridor, a depression several hundred kilometers in length. The most promising plan (K. V. Dolgoplov and E. F. Fedorova, *Voda—Natsional'noe Dostoyanie*, Moscow, 1973) envisages a canal from the confluence of two tributaries of the Ob', the Irtysh and Tobol, which meet at Tobol'sk, to

Atrek, close to the Iranian frontier—a distance of some 2300 km. This would allow up to 50 km³ of water to be directed southwards annually.

The main engineering problem would be posed in crossing the Siberian-Asian watershed, which would involve pumping the water to a height of some 75 m. The cost of the pumping would be considerably reduced by allowing the water to drive hydroelectric turbines on its downward gradient. An announcement at the Moscow Geographical Congress indicates that the canals are also to be of navigable depth, which would favour the ultimate cost-effectiveness of the project. Several giant reservoirs (colloquially called "seas") with areas up to 100 km² would be constructed along the Ob', Irtysh, and Tobol rivers to store the water before transmission.

A number of variants of the plan have been worked out. The canal itself could be continued through to Khanty-Mansiisk at the confluence of the Irtysh and Ob', or else (as Dolgoplov and Fedorova show it), water from the Ob' could be forced back up the Irtysh to Tobol'sk by a series of pumping stations. The headwaters of the Ob' and Irtysh are to be joined by a canal already under construction. A sidebranch of the Tobol'sk-Atrek canal could be taken westward over the Urals to feed the Ural river which has insufficient water for the massive irrigation projects planned for its lower reaches.

Such plans have caused considerable outcry among conservationists throughout the world, who fear that a diminution of the inflow into the Arctic Ocean might cause massive environmental changes and even result in a southward spread of the polar icecap. To ecologists not of the doomwatch persuasion, however, such an outcome seems unlikely. The hydrography of the Ob' basin has been extensively studied. The average annual discharge of the Ob' at Khanty-Mansiisk is some 233 km³, almost five times the proposed uptake of the canal. At Salekhard, where the river flows into the Ob' gulf, the annual discharge is 400 km³. The Ob' basin already has a highly developed economy, including the inland port and industrial complex of Novosibirsk and the vast gas and oil fields of Tyumen', Urengoi and Samotlor. Hydroelectricity from the lower Ob' is a fundamental factor in the planned development of these deposits. The Ob' and its tributaries are, moreover, important fish producers.

As even the plans for the lower Ob' hydroelectric station were preceded by a detailed survey of the microclimate of

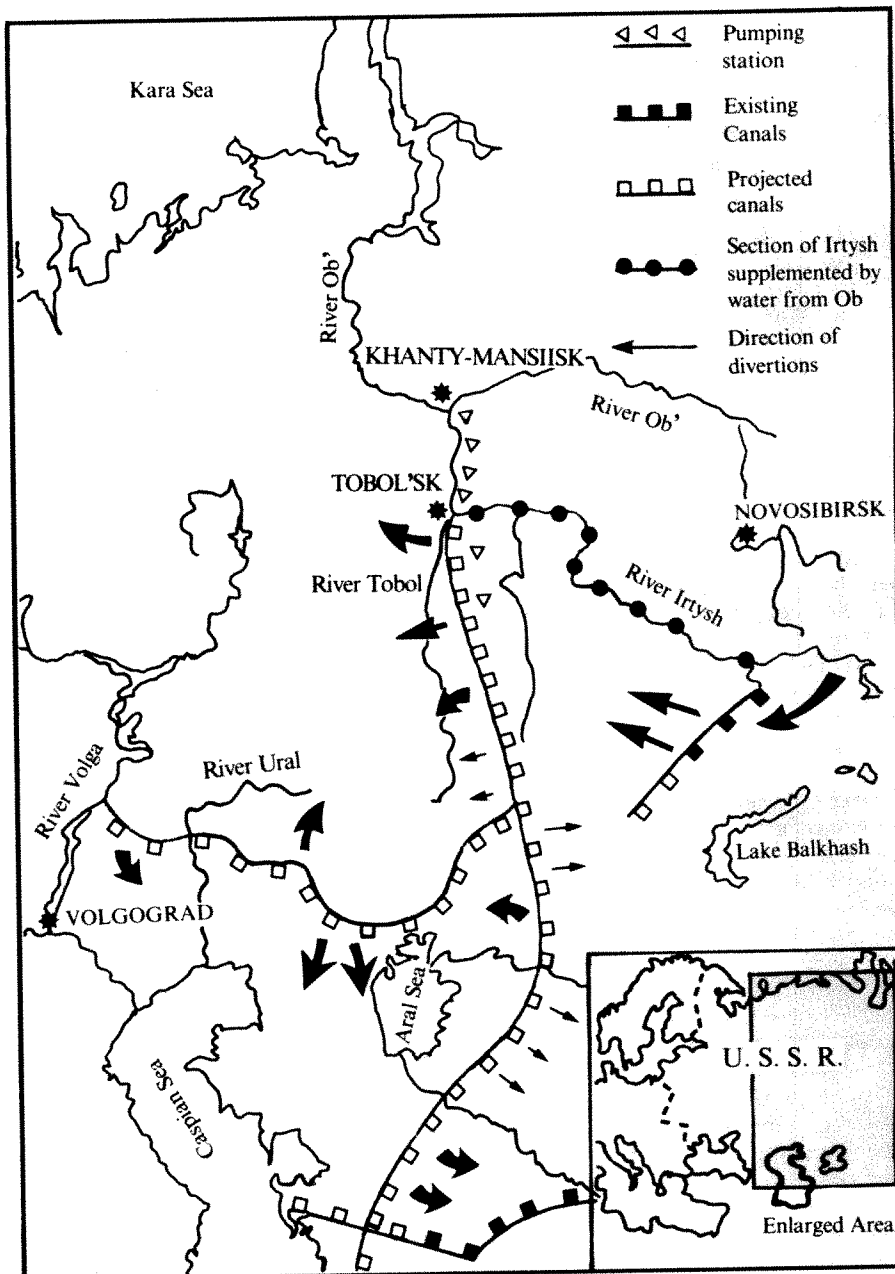
the area, and as the exploitation of the Arctic coast of Siberia is also an important feature of Soviet planning, it would seem highly unlikely that the effect of such proposed diversions would not be investigated as fully as possible. According to the *Novosti* agency, southward redistribution of water from the Irtysh and Ob' (some 75 km³ when all the canals are operational) "would not cause the slightest damage to Siberia". In view of the stress on possible environmental effects written into the resolution this statement may seem somewhat premature, but the emphasis on ecological hazards is significant.

Constructing the canals

Less concern abroad has been evoked by the methods to be used in constructing the canals. Early "spectaculars" of Soviet hydroengineering such as the White Sea Canal were excavated largely by shovel-power and a labour force of "socially undesirable elements" undergoing re-education, but the new canals can command a higher technology—in particular, nuclear explosions. Indeed, such explosions have already been used on another river-diversion scheme west of the Urals which will bring water from the Pechora south to the Kama and thence to the Volga and the Caspian Sea. According to one of the few press articles dealing with this project (it appeared in *Krasnaya Zvezda*, the Red Army newspaper, in 1970), the first stage of canal should be operational by the end of the decade and, with its "cascade" of hydroelectric stations, should recoup all costs within four years.

In view of the fact that, according to Moscow Radio, work on the main canal and reservoirs of the Ob' scheme will not commence until the 1990s, it is interesting that the chief surveyor of the Pechora project, Viktor A. Chistyakov, who had previously worked on the Irtysh-Karaganda canal and the Middle-Enisei hydroelectric station, considered that the terrain of the Pechora project was incomparably more difficult. It is the Pechora diversion which nevertheless seems to have the priority.

The use of nuclear explosives is a feature of the Pechora diggings. The criterion for choosing nuclear rather than conventional blasting is primarily cost-effectiveness; a nuclear charge is more efficient when an explosion of more than a few kilotonnes is required. The Soviet Union has shown an active interest in peaceful nuclear explosions (PNEs) since 1960, when she suggested that a thermonuclear explosion might be used to obtain fresh water from glaciers (the suggestion was not implemented). By using a method of deep-drilling and subsequent collapse, radio-



active products can be confined far below the actual installations, so that there seems to be no great safety-hazard; indeed, a film of an early PNE which formed a crater to be used as a reservoir included shots of a man swimming in the crater only a few days after the blast. (No information was ever released about his subsequent history).

Unlike the United States, which is interested in PNEs principally as a means of gas stimulation and envisages no major construction projects that could possibly employ PNEs, the Soviet Union has in its river-diversion schemes a field where they might play a useful role. It is not surprising, therefore, that the Soviet Union is very interested in the conclusion of a threshold treaty which would define the size and amount of charges to be fired and the conditions under which they may be employed. The proposed treaty, now awaiting

ratification by the US Senate, suggests a maximum charge of 150 kilotonnes, a size which is easy to verify seismologically and which could not be confused with any unauthorised weapon test. If more than one maximum blast is to be used in a single row-charge, a maximum of 1.5 megatonnes is proposed for the row; on-site inspection by the other contracting party would be permitted and, furthermore, that party would be permitted to have monitoring instruments down each borehole to measure the yield. This is the first time that the Soviet Union has expressed a willingness to permit on-site inspection, and may well be an indication of how vital the new canals are to the future Soviet economy. Perhaps the new treaty, with all its political implications, may prove no less a benefit to the canal scheme than the immediate practical gains of irrigation and hydroelectric power. □

At a standstill after a year

Peter Pockley looks at Australian science after 12 months of Mr Fraser's government

NOVEMBER 11, long celebrated in Australia as Remembrance Day to commemorate the end of the First World War, has now been endowed with a second significance. The day was marked in 1976 by large crowds expressing their continued opposition to the manner in which Mr Malcolm Fraser and his Liberal/Country Party coalition persuaded the Governor-General, Sir John Kerr, to sack the Labor Government of Mr Gough Whitlam only two hours after Sir John had laid wreaths on the eleventh hour of the eleventh day of the eleventh month of 1975. Mr Fraser pulled this crisis on Australia, and one month later won the election, on the justification that Labor could not manage the economy.

On his first anniversary in office, Mr Fraser presided over a stagnant economy with his principal target of curbing the 12–14% inflation as much out of range as it was under Labor, with an unemployment rate of about 5% that is the highest since the 1930s and with worse to come as school and university leavers flood the job market in December and January, and with an even worse industrial record than Labor's. Yet his government remains surprisingly popular, a fact which many observers put down to the success with which the release of information and the opportunities for newsmaking debates have been carefully controlled to the point of almost obsessive secrecy. Last weekend's heavy 17½% devaluation of the Australian dollar could dissipate some of that popularity. The Labor Opposition, however, is only just emerging from the shock of its demoralising defeat.

Effect on science

This is the context within which the first Fraser Budget and its effect on science must be assessed. With the one exception of grants for medical research, the effect on science generally has been to impose at least one year of treading water, and quite possibly several years of struggle to stay afloat. Fraser and his Treasurer, Mr Philip Lynch, were determined to tackle inflation principally by drastic cuts in public spending, and their scythe cut deeply into the flesh of all public instrumentalities; unfortunately, most were already lean after cuts by Labor in 1975, and the bones are now exposed.

Take CSIRO, for instance. Its Jubilee year of 1976 has been scarred by a

Budget allocation for 1976–77 of \$131 million (1.6 Australian dollars = £1) which does not even cope with inflation since the 1975–76 allocation was \$123 million. The government claimed the CSIRO's "activities will be held to the same real levels as in 1975–76", a statement greeted with some cynicism by CSIRO administrators as they go about their cheerless task of cutting staff. The foregoing quote from the Budget paper was preceded by confirmation of "an independent external inquiry into the operation of CSIRO". This inquiry is now underway, but as the Budget said, "In the interim CSIRO's activities will be held . . .". It's going to be a hard last period for CSIRO Chairman Sir Robert Price (formerly Dr Jerry Price) and for his unnamed successor who should take over in March 1977.

The 19 universities of Australia are the next greatest sponsors of research. Their growth has been held and there are great uncertainties about the future. Through the Universities Commission, the universities will receive \$562 million for the calendar year 1977, as compared with \$542 million for 1976. The Education Minister, Senator John Carrick, has claimed this constitutes a 2% real growth rate, but no university administrator has publicly agreed; many are trumpeting "hard times ahead". The situation in universities for funding new equipment and replacing old is marginally better than last year, but the real crunch comes in the provision of staff. "Freezing" of establishments and "natural wastage" are the order of the day as universities face unavoidably increasing costs from salary rises as staff grow older on average and are not balanced by equalising intakes of young, cheaper lecturers.

The two principal sources of government money for supporting the research of individuals and small groups, largely in universities, appeared to fare better in the Budget than ever. This was a shrewd, and essentially very cheap, way of the government preventing the kind of damaging outrage which greeted Labor's cuts in these funds in the previous year's Budget. In reality, though, the Australian Research Grants Committee (ARGC) received \$9.1 million, a shade higher only than the previous year. The National Health and Medical Research Council (NH&MRC) was the only group smiling as it received \$9.1 million, a significant increase on \$5.2 million last year; this



Mr Fraser, 12 months on

was a tribute to the success of the public lobbying by the medical researchers, which was not matched by those in the natural and social sciences and the humanities serviced by ARGC.

Individual researchers in academia showed last year how well they could protest at threats to their personal support, but in fact they are far more dependent on maintenance of the overall funding received by their institutions and that is decidedly unpredictable. This is because the government has replaced the triennial system of forward funding of universities with, in essence, an annual budgetary arrangement masquerading under the description of "rolling triennium". Significantly also, the previous triennial forward commitments for ARGC and NH&MRC were quietly dropped and only one-year funding substituted.

The Australian Atomic Energy Commission, under its new part-time Chairman, Professor Don George, faces the year with \$21.1 million, radiated up only \$1.1 million on last year. The Antarctic Division of the Department of Science has been frozen at \$6 million. Industrial Research and Development Grants, administered by the Department of Industry and Commerce, have not developed at all—they have dropped by \$3.9 million to \$15.4 million. Labor's Department of the Environment was swallowed into the maw of a ragbag department called Environment, Housing and Community Development with the deliberate intent of spending as little federal money on environment protection and research as possible. The whole department suffered a shattering 37% decrease in funds and the environment part of it did not even rate a mention in the main Budget documents. Defence science and technology received \$89 million (up \$5 million) out of a total defence vote of \$2,178 million, the only trouble being that nobody really knows what this will be spent on.

Science Department come-back

Reports in these columns earlier this

year chronicled the tribulations of the Department of Science during the first few months of Fraser, when the very continued existence of the Department seemed unlikely. With some good training beneath their skins for survival gained when the Labor Party came to power, the senior staff of the Department knew that if they could survive the first six months of the cost-slashing Liberals they would coast into the period when the new Cabinet aged rapidly as they had to cope with problems of their own making and could no longer blame their predecessors.

This stage has now arrived. But the Department had good reason to be concerned for its future. Its Minister, Senator James Webster, was 25th out of 25 in seniority and his voice was weak in the face of possible Cabinet reshuffles. The Science Task Force of

the Royal Commission on Australian Government Administration had strongly recommended dispersal of the Department's operational divisions among other departments, and there was much support for this view among the scientific community. Fraser had put a hatchet man among the public service to search for and destroy "wasteful and extravagant expenditure", and science was one of those on the block. And there were severe tensions between the Canberra head office and some operational divisions.

In the long run, the hatchets proved blunt and the only waste was the cost of the blades and the unpublished report. The Royal Commission did not come out with a specific recommendation on the Department, and the Secretary, Sir Hugh Ennor, walks taller and looks set to see out his full

term to retirement next year.

Senior members of the Department are setting out to win friends they've not previously had in the universities, at the same time as the Australian Science and Technology Council (ASTEC), a potential rival to the Department in the high stakes of policy formulation, is working hard on its own survival. The Department's data collection outfit, Project SCORE, has its statistics on national R&D expenditure and manpower for 1973-74 at press; official, private inquiries into aspects of research organisation are planned.

The Australian preoccupation with organisations, structures and the dreary politics which go with them to the exclusion of concentration on the substance of research has again been amply demonstrated in Fraser's first year. *Plus ça change* . . . □

CANADA

Reed in the wind

An agreement by the Ontario government to give a paper company the right to cut timber and build a mill in the largest area of the province ever proposed for such a purpose has caused a storm in the provincial legislature. David Spurgeon reports from Ottawa

THE recent controversial agreement between the province of Ontario and Reed Paper Limited covers an area of almost 19,000 square miles, larger than the entire province of Nova Scotia. Although it is subject to the outcome of environmental impact studies and hearings—which, according to the premier, William Davis, will take 2½ years and could still leave the province free to refuse the licence—that has not silenced the critics.

Prominent among them are environmentalists and Indian leaders, and they are led by New Democratic Party leader Stephen Lewis. The "memorandum of agreement" between the province and the company was signed just in time for it to be tabled on the first day of the Ontario legislature's autumn sitting on October 26, and was met by a concerted attack from Mr Lewis. He produced an internal memo from the government's Environment Ministry indicating that some of its own foresters believed the ministry had failed to enforce proper woodcutting practices. The government later said the memo, which warned of a timber shortage by the year 2000, contained only individual foresters' opinions.

The government said the company must submit to a full hearing by the environment board, which heretofore

has dealt only with government projects. All interested parties in the area, such as Indian groups, will be heard by the board. Before that, the company must make public its proposals for a comprehensive forest management plan for its mill, and if the project goes ahead the company must pay back to the government the costs of the studies of timber potential in the area. The agreement expires by January 1980, if the plans are not approved by then.

One of the reasons for the furore is that Reed is the company which owned the chemical plant that was believed to be responsible for mercury discharges into the English-Wabigoon River system, which disrupted life for hundreds of Indians and raised the spectre of Minamata disease among them. The agreement involves some of the same Indians who were previously affected by the mercury poisoning. While those in the area are flatly opposed to the development, which was initiated without consulting them and which would, in the opinion of some, threaten their livelihood, local white residents feel quite differently.

The proposed development would cost about \$400 million and provide up to 1,200 new jobs. It would bring new economic life to the area, whose residents for many years have suffered the uncertainties involved in living in this isolated region. One community is Red Lake (population 2,300), a departure point for rich sportsmen who fly into the area for hunting and fishing. It is also the central housing point for workers in the largest industry in the area: gold mining.

Mr Davis has pointed out that many of those who oppose the Reed develop-

ment have never visited the area and wouldn't know the difference between a balsam and a jack pine. Red Lake Reeve John Goodwillie sees the paper mill as the basis for an economy other than tourism or the vagaries of gold mining. For years the town has seen mines closing and opening, lay-offs and hirings, and market fluctuations. And many agree with him that the mill is a potential boon—not something to be feared. As for the mercury incident, Goodwillie says that as far as he's concerned, "it wasn't Reed that started polluting Dryden, it was a small company and the only thing that Reed did was buy them out and get stuck for cleaning it up."

Ontario's Minister of Natural Resources, Leo Bernier, has in fact been hinting that the company "might just walk away" from the project because of the criticisms. Both Ear Falls and Red Lake councils have passed resolutions supporting the proposed development.

Some see the controversy in a different light. Mr Bernier has admitted, when questioned, that the province is 1½ years behind in its reforestation programme. A paper prepared by an employee of the Natural Resources Ministry raised the spectre of huge acreages going out of production annually as a result of present management practices. It also accused Reed of uncontrolled cutting of Boreal softwoods and partial cutting of high quality stands of Boreal mixed woods.

Public pressure may now force a much more active scrutiny of the province's dealings in many areas, since for the first time a private project in Ontario will have to meet economic tests and numerous environmental, social and cultural tests, too—all of it in public. □

USA

Castles in the air

Colin Norman reports from Washington on a heavily-criticised study from the Environmental Protection Agency (EPA)

A MASSIVE study of the health effects of exposure to low levels of air pollutants, conducted by the EPA at a cost of some \$22 million, is so riddled with errors and shortcomings that the results produced so far are virtually useless. That verdict, reached by a team of investigators working for two Congressional subcommittees, is likely to prove more than a mere embarrassment to EPA, for the study was originally expected to provide the basis for the agency's air pollution regulations.

The study, known as the Community Health and Environmental Surveillance System (CHESS), was an ambitious effort to collect and compare data on the levels of pollutants and the incidence of a variety of health problems in six US cities. Data collection began in the late 1960s and ended last year, and the first results (covering information collected in 1970 and 1971) were published in the form of a monograph in May 1974. The monograph purported to show that there is an association between various health problems, such as the incidence of asthma and heart and lung disorders, and levels of sulphur dioxide in the atmosphere.

Though such a correlation is not exactly surprising—the London smogs of the 1940s and 1950s had provided a rather graphic demonstration of the association between high levels of pollutants and severe health problems—the CHESS findings and the methodology used in the study have encountered considerable scientific criticism.

Most of the criticism has been concerned with the methods used in gathering and interpreting the data, but last February the study was dealt a severe new blow when the *Los Angeles Times* published an article alleging that the finding in the first CHESS monograph had been deliberately distorted by an EPA official "in an effort to prove that pollution from sulphur-bearing fuels has an adverse impact on human health". The article implied that the findings had been distorted in order to provide scientific justification for EPA's air pollution regulations.

The allegations set off a stampede of lobbyists from electricity companies, coal producers and other industries to Capitol Hill, to urge that no new

regulations be adopted at least until the CHESS study has been examined. The matter was urgent because Congress was then about to consider amendments to the Clean Air Act, and consequently two subcommittees conducted a public hearing to examine the charges. The hearing produced a score of witnesses, most of whom flatly denied that any deliberate distortion of the data had taken place. But the hearing did not go deeply into the methodology behind the CHESS study or the validity of the data. For that purpose, a Congressional staff investigation was launched, under the guidance of Congressman George E. Brown Jr, and with the help of a battery of scientific consultants. It was the result of that investigation which was published last week.

The investigating team reported that the CHESS study provides "a picture of a program pressured by EPA management-imposed time constraints to meet legislated mandates for promulgating new standards, hampered by inadequate mechanisms to detect and correct technical problems, and handicapped by budgetary and management restrictions placed on the program after it was well under way". The upshot, Brown said last week, is that the CHESS results published so far "have virtually no quantitative value".

Many of the problems stem from the fact that atmospheric measuring techniques, used particularly in the early stages, are too imprecise to yield good information. For example, an attempt to see whether there is a threshold

level of sulphur dioxide pollution below which health effects are not experienced was hampered by the fact that the measuring technique could not detect concentrations of the pollutant below $25 \mu\text{g m}^{-3}$, and the uncertainty in the measurements sometimes exceeded 100%.

Most of the CHESS data is still to be analysed, but the Congressional report argues that "there is serious doubt that the analysis even when completed will ever be sufficiently credible to support the stated objectives of the program". EPA has, however, initiated a new monitoring program, known as CHAMP (Community Health Air Monitoring Program), which the Congressional report calls "clearly an improvement in aeronomic pollution measurement", but it won't yield health data because at present it is not coupled with a health monitoring programme.

In other words, regulation of air pollution will continue to be based on imprecise information about the effects of low levels of pollutants on human health, a fact which is sure to be exploited to the full by purveyors of sulphur pollutants. They won't have to wait long for an opportunity to state their case, because when Congress returns in January one of its first items of business will be a reconsideration of the Clean Air Act (no agreement was reached last session on proposed amendments to the act, and the matter will be brought up again next session).

It should be noted, however, that although the CHESS study has provided no scientific basis for the present air quality standards, it has provided no reason to reject them. □

Warning on fluorocarbons

Over the shrill objections of the cosmetics industry, the Food and Drug Administration (FDA) and the Consumer Product Safety Commission (CPSC) last week proposed separate actions to reduce and eventually eliminate the use of fluorocarbons in aerosol spray cans. The FDA's proposal, which would involve placing a warning label on fluorocarbon-containing products, was expected; the CPSC action came as a surprise.

FDA has authority over products such as hair sprays, deodorants, perfumes and anti-perspirants, which account for some 80% of the total release of fluorocarbons into the atmosphere. The action announced last week will require the following statement to be printed on the label of aerosol spray cans containing

fluorocarbon propellants:

Warning. Contains a chlorofluorocarbon that may harm the public health and environment by reducing ozone in the upper atmosphere.

No date has yet been set for the regulation to take effect, but barring legal action by the industry to block the proposal, the warning labels will probably be required early next year. The action, noted FDA Commissioner Alexander Schmidt, is only an interim measure—FDA will soon propose a timetable for phasing out all non-essential uses of fluorocarbons entirely.

The CPSC action came in the form of a 5-0 vote by the commissioners to ban use of fluorocarbons in aerosol products not regulated by FDA. No timetable was set by CPSC for carrying out the ban, however.

BRITAIN

Another delay for BNFL

Gillian Boucher reports on the latest developments in the controversy over the reprocessing of nuclear fuel

JUST when they seemed about to gel British reprocessing plans have received a vigorous stir from Mr Shore, the Secretary of State for the Environment, who announced last week that planning permission for expansion at Windscale was to be withheld until he had given it more thought. Cumbria County Council had given British Nuclear Fuels Ltd (BNFL) a provisional go-ahead on November 2 but as the application was outside the scope of the county's development plan it had to be referred to Mr Shore.

If he had done nothing the application would have received automatic approval after three weeks. It was agreed at an inter-departmental meeting on November 15 that Mr Shore would not call in the plans. On the last day on which he could intervene he said he needed more time; by last weekend the House of Commons Select Committee on Science and Technology was considering whether to take up the issue and question Mr Shore itself.

Mr Shore has two choices: handing the matter back to the county council, which virtually means accepting it, as the county council was "minded to approve" the application; or calling it in for his own decision, which will probably mean either a public enquiry or a Planning Inquiry Commission and a delay of at least a year.

BNFL wants to expand and update its Magnox reprocessing plant, develop the process of vitrification of nuclear

waste, and build a new "Thorp" plant for reprocessing oxide fuels. It is this last which arouses such passion because of the large amounts of foreign fuel that it would reprocess. Although it means 1,000 new jobs and around £500 million in earnings from deals with Japan, Germany and Sweden already in advanced stages of negotiation, it also carries the dangers inherent in purifying and transporting plutonium. Friends of the Earth feel that the plutonium peril is so great that oxide fuel should not be reprocessed; but a BNFL spokesman says that although oxide fuel is not as susceptible to corrosion as Magnox fuel, corrosion and leaks into the water in storage tanks will eventually occur if the fuel is not reprocessed.

Mr Shore probably "decided not to decide" as a result of strong pressure for a public enquiry from environmentalist groups. But what he wants more time for is not at all clear. He may want to talk to more specialists or learn the views of Mr Carter. Some critics fear that he is merely waiting for the interest built up over recent weeks to die down before giving his verdict.

He has reserved his decision on the whole of the planning proposal, not merely the oxide plant. But the reprocessing of Magnox fuel cannot be delayed and according to BNFL a refusal on that would result in the premature closure of the Magnox nuclear power stations. A decision on the oxide plant is not as urgent because spent oxide fuel can be stored, but Britain already has two oxide-consuming AGR reactors in action. A ban on oxide reprocessing would force Britain

to take its oxide fuels elsewhere for reprocessing and mean the loss of potential foreign business.

Though nobody would deny the gravity of the problems involved, BNFL is understandably irritated that the government should be stalling 8 months after giving it permission to seek foreign deals—permission which was supposed to have been preceded by adequate debate of the issues. And J. C. C. Stewart, Deputy Chairman of the Nuclear Power Company, said at a British Nuclear Forum Conference last week that it would be "absolutely appalled if the matter did not go forward".

One possible further hurdle, though, lies in the financing of the oxide plant. Last July the government approved BNFL's proposed investment in the Magnox fuel plant and vitrification development, but it has not yet decided whether to act as the lender of last resort for the oxide plant. Most of the finance, however, would come from prepayments from the foreign countries with whom the deals are being negotiated.

Meanwhile the Cumbria council itself may not be allowed quickly to forget its decision. Friends of the Earth and the Lawyers' Ecology Group believe that it behaved improperly in not considering such aspects of the application as the transport of nuclear fuels and the build-up of radioactivity. Mr Shore had written to the council confirming that these were matters for the government, but according to the Lawyers' Ecology Group this does not absolve the county council of its duty to consider the questions. Several pressure groups are likely to come together to take legal action against the council if the planning application is not called in. □

Animal anxieties

BRITAIN'S Medical Research Council (MRC) found it necessary to release a statement last week in an effort to quell a growing storm at its Laboratory Animals Centre at Carshalton, Surrey. The row is over the MRC plan to charge the 70 animal breeders participating in a 26-year-old accreditation scheme the full economic costs of the £70,000 service provided.

The Council said it reaffirmed the importance it attached to its policy, but said it "has been made aware" of the problems in relation to charging for the scheme and had therefore set up a committee with the "necessary powers to act" to review these urgently. It is thought that the committee could report in weeks rather

than months.

Last week, however, delegates from nine trade unions represented at the Carshalton centre called a press conference just as the Council itself was about to meet. They said that if as they expected the Council decided to set up a working party they would have no confidence in this as it was "not a way to proceed". Industrial action was being contemplated, and one union had already asked the Minister of Education and Science to intervene.

The scheme is voluntary and consists of grading, by their degree of freedom from microorganisms, some 3 million animals used every year for research and teaching in labs up and

down the country. The unions say the scheme is now in danger of breaking down totally because the majority of breeders, apparently unable to afford the charges, are threatening mass resignation.

This, they say, would result in "the loss of public control over the commercial breeding of laboratory animals" and could cost the community more than it saves insofar as the use of non-accredited animals grows and leads to costly outbreaks of disease that might not otherwise occur. There is no clear sign, however, that the MRC actually wants the scheme to end, and it seems likely that the new committee will seek ways to maintain it while introducing charges.

Chris Sherwell

IN BRIEF

Environmental warfare action

The Natural Resources Defense Council (NRDC), an environmentalist organisation, has gone to court in an effort to block ratification by the United States of an international treaty prohibiting some types of environmental warfare. NRDC lawyers have argued that since the treaty, which is now being considered by the United Nations General Assembly, would exclude only environmental modification techniques which may have "wide-spread, long-lasting or severe effects", it would not outlaw use of such techniques as cloud seeding and other potentially more damaging environmental modification efforts. If the treaty is ratified, NRDC asserts, it would serve only to "legitimise the use of weather modification and defoliants as weapons of war".

Similar criticisms have also been ex-

pressed by countries including Japan, Sweden, Pakistan and Yugoslavia. NRDC is seeking a novel way to block US ratification of the treaty: it is arguing that the National Environmental Policy Act requires the State Department to file an environmental impact statement setting out the risks and benefits associated with the treaty, and until such a statement has been published, NRDC is seeking an injunction to prevent US ratification.

Windmill site chosen

A small town in New Mexico has been chosen as the site for the world's largest electricity-generating windmill. The Energy Research and Development Administration (ERDA) last week announced that a 200 kW wind turbine generator will be constructed next year in Clayton, a small town with about 3,000 inhabitants in a windswept

region of the Great Plains. The turbine, which will be field-tested for two years, will be the first wind turbine to be constructed in more than 30 years for generating electricity for public consumption in the United States. It will be followed in the early 1980s by two 1.5 MW generators in other, as yet unannounced, locations. Designs for those two generators are now under development by ERDA and, when constructed, they will be the largest wind generators ever built.

Bomb at Swedish plant

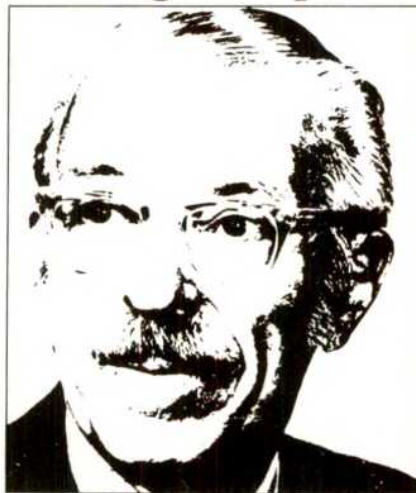
A 25 kg bomb at the Ringhals nuclear power station on Sweden's west coast was discovered last weekend after a Göteborg newspaper received an anonymous letter. The bomb was apparently too far from the reactors to have damaged them but could have damaged power lines and transformers.

THE media are providing news of the furbish lousewort (*Pedicularis* sp.). This is a scrophularious plant that is not yet on the endangered list, but is about to receive its diploma in this category, come 1977. Louseworts were so named in England because it was alleged that "the cattell that pasture where plenty of this grass groweth become full of lice". It seems that about 30 furbish louseworts were discovered in the juggernaut pathway of a new federal electric power dam in a remote corner of northern Maine. Such an event sets up two groups: good guys (lousewort lovers) and villains (power dam protagonists) on opposite sides of a "two-valued orientation". The law does not permit a compromise of moving lousewort clusters to another niche in Maine.

Nostalgically I recall that more than twenty years ago I wrote a short article deploring the march of high tension lines through the wild Ramapo mountains, a home of white-tailed deer, mountain laurel, rattlesnakes and blueberries, only 30 miles north of New York City. Like most protesters, I was then, as now, a great consumer of electric power. I am also a lover of the more common louseworts, whose curly fronds rise in reddish sprouts from the wet mud as soon as the snow melts in high mountain meadows. Later they will unfold their small luminous pink flowers called "elephant heads".

It is important to preserve endangered species, but whether we can preserve their present habitats may be doubtful. Sometimes the niche is at greater risk than the species, which can be transferred elsewhere, as with

some of the noble silva of California. The giant sequoia is indigenous in only a few groves on the western slopes of the Sierra Nevada. Yet its lovers have carried it to distant parts; it towers above native trees on the shores of the Vierwaldstättersee in Switzerland. Specimens of its cones

Endangered species

THOMAS H. JUKES

and branches first reached Europe in 1853, and now two giant sequoias in the garden of a palace in Spain are presumably the tallest trees in Europe. If cherished, they can increase in stature for two thousand years. The coast redwood, a similar species, is probably regarded widely as being endangered, but actually it is only the large specimens that are at risk; the species itself is vigorous and thriving. A two-day hike through the Cali-

fornia coast range near Big Sur impresses one that there are probably, and fortunately, more redwoods (small ones) than people in California.

Surely the skills of horticulturists and plant scientists should be increasingly summoned to preserve endangered plant species. Cloning, asexual propagation and transplanting can all be used. The problems are different for animals. Much praiseworthy effort is currently taking place in zoos to save rare and dwindling species of animals, but, when preserved, where will they live? The inherent behaviour of an individual species towards people may either favour or endanger its survival. In the 1920s, the ornithologist Dawson said that there were 20-odd giant condors left in the USA and that one of their principal enemies was "scientists". If the 1920 figures were correct, the numbers of this bird have not since diminished, but it seems to have lost its ecological niche because of human intrusion, and, perhaps, diminution of food supply. Its smaller relatives, the vultures, are in contrast highly adaptive and proliferative. They even eat carrion on the roads in the form of dead animals killed by highway traffic. But the shy and lonely condor will have none of such contact with the doings of humans.

No regrets have yet been voiced over what we are told is the impending extinction of the smallpox virus. Doubtless this little piece of DNA helped, years ago, to keep the human population curve from ascending too steeply. But admirers of smallpox will find few sympathisers.

news and views

Is HnRNA really pre-mRNA?

from Bob Williamson

THE central dogma (and much experimentation) convinces us that genetic information in animals and plants is encoded in nuclear DNA, copied into messenger RNA and then translated into proteins in the cytoplasm. Therefore there has to be an intermediate, a nuclear RNA molecule (the primary transcript) which includes mRNA sequences and which is processed into the cytoplasm. This idea, postulated and elaborated by Scherrer and Darnell in particular, has been widely accepted for many years, yet is supported by remarkably little evidence.

A large proportion of the RNA isolated from nuclei consists of large molecules some 10 to 20 times the size of mRNA. This is particularly true when pulse-labelled RNA, which has incorporated radioactive precursors for a matter of minutes, is examined. Much of this large nuclear RNA (known for many years as heterogeneous nuclear RNA, or HnRNA) falls apart when denatured, demonstrating that it is nicked and held together by hydrogen bonds—or perhaps is aggregated *in vivo* or during isolation.

The paradox of the rapidly labelled HnRNA is that, as Henry Harris pointed out 15 years ago, the great majority of it is broken down in the nucleus and never reaches the cytoplasm at all. Cytoplasmic mRNA accumulates very slowly, and no one had convincingly demonstrated a precursor-product relationship between HnRNA and mRNA using radioactive labelling. The major problem has been the mixture of different RNA species present in nuclear RNA. Even in erythroblasts, where the protein synthesised is mainly haemoglobin, less than one part in a thousand of the nuclear RNA is globin mRNA—and the nature of the other 99.9% is unknown. Attempts made by Scherrer and John Bishop in particular to assay for specific mRNAs using labelled cDNA demonstrated the presence of globin mRNA in large duck nuclear RNA, but in such small amounts as to raise some doubts as to possible contamination or spurious cross-hybridisation. These doubts were

stimulated by the fact that McKnight and Schimke (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 4327; 1974) in a very careful study, failed to find a nuclear high molecular weight precursor for ovalbumin mRNA, as did Lizardi (*Cell*, **7**, 239; 1976) for silk fibroin gene transcripts. The doubts were summarised recently in a review by Davidson and Britten (*Q. Rev. Biol.*, **48**, 565; 1973).

The most recent study on globin nuclear RNA (in this case from mouse foetal liver) again demonstrates a small amount of "precursor" double the size of the 9S mRNA (Ross, *J. molec. Biol.*, **106**, 403; 1976). The half-life of the precursor is approximately 45 min. No precursor more than double the size of the mRNA was found using cDNA to hybridise across a 98% formamide denaturing gradient. The undoubted experimental care shown by Ross only serves to emphasise that this type of experiment cannot extend our information about HnRNA beyond Scherrer's conclusions of 5 years ago—the "noise" due to other sequences is just too great.

Advances in this field really depend upon the isolation of specific nuclear RNAs containing only a single mRNA or restricted group of mRNAs. With a defined precursor many questions could be answered: the size of the primary transcript, the significance of the many sequence features (oligo(U), oligo(A), double strand regions and palindromes), the arrangement of unique and repetitive transcripts along the length of the HnRNA, and even the position of the mRNA could be studied. (These cannot be determined now because one has no way of knowing whether the average features of the mixture of HnRNAs really relate to specific mRNA precursors or not).

Several groups have been trying to isolate globin-specific nuclear RNA, and the first report to reach print is that of Curtis and Weissmann (*J. molec. Biol.*, **106**, 1061; 1976). They have used a particularly elegant column chromatography technique which was first developed for the isolation of viral nucleic acid sequences,

and should have wide applications for any mRNA or DNA where a large amount of complementary probe is available. The probe in this case was complementary DNA (cDNA) prepared with viral reverse transcriptase, and then elongated with terminal transferase to put on a poly(dC) tail. This behaves just like 'ordinary' cDNA when hybridising to messenger RNA, or the mRNA sequences in nuclear RNA. Therefore, since globin cDNA was used, it identified and paired with globin RNA sequences only, and with the expected kinetics in solution.

The hybrid, of course, now has a poly(dC) tail. This will bind to poly(G) by specific hydrogen bonds. In practice a close relative of G, inosine (I), which has the same pairing properties, was used. With a poly(I)—Sephadex column only elongated cDNA molecules containing poly(dC) were retained. These will, of course, include any that have formed hybrids with globin-specific nuclear RNA molecules.

It was no surprise to find that much of the globin-specific nuclear RNA in the mouse erythroid Friend cell line is approximately the same size as mature, cytoplasmic globin mRNA. However, when RNA pulsed for 20 min was examined, an additional peak at double this size (approximately 500,000 molecular weight) was also found. This had previously been found in duck erythroblasts and mouse foetal liver as well. Again, little material larger than 15S could be isolated after denaturation. Curtis and Weissmann conclude that their results definitively demonstrate a nuclear precursor RNA containing globin mRNA at least double the size of the mRNA itself. A number of controls, using fingerprint analysis and labelled cytoplasmic globin mRNA, demonstrated both the specificity of the method and the absence of aggregation.

The most important aspect of the Curtis/Weissmann experiments, however, is their wide applicability to all animal and plant nucleic acid systems. At the moment, progress is difficult because of the large numbers of genes

and transcribed sequences. There are some 10 million gene-size pieces in the human genome, and at least 1% of these are transcribed in the nuclei of even the most differentiated cell types, albeit at low levels. The affinity chromatography tricks developed here, and similar ones using cDNA covalently bound to cellulose columns (for instance Levy and Aviv, *Proc. natn. Acad. Sci. U.S.A.*, **15**, 1844; 1976), allow the isolation of one specific DNA or RNA sequence from this plethora.

The emphasis during the past few years on covalent integrity of the nuclear RNA (which has led to so much formamide and DMSO technology in an attempt to denature aggregates) does not answer the real point. It would be quite possible for the very large precursor to be nicked during or shortly after transcription, in which case a small mRNA-containing molecule would be obtained. In fact, this obviously does happen, in the course of normal processing to the cytoplasm. It is most instructive to compare these data on isolated RNA molecules with the sizes of primary transcripts seen with the electron microscope.

Charles Laird and his colleagues (Foe, Wilkinson and Laird, *Cell* **9**, 131; 1976) studied electron micrographs of

chromatin-associated fibre arrays of nuclear ribonucleoprotein from milkweed bugs. The non-ribosomal regions of the chromatin had far fewer "growing RNA chains" than the nucleolar regions, but these still could be measured. Each "active transcription unit" has a defined "start point" (showing directly that there is a specific initiation point for non-ribosomal gene transcripts, in itself a finding of some importance). Moreover, the average size of a non-ribosomal RNA primary transcript is approximately 8 million daltons, very nearly the size originally predicted using non-denaturing gradients. Unfortunately, Laird cannot yet distinguish individual mRNA precursors in the electron microscope.

As for so many other problems in molecular biology, DNA recombinants will enable these questions to be resolved. When a genomic DNA sequence containing the globin gene is isolated, it will then be possible to follow the fate during processing of transcripts of the sequences adjacent to, and progressively further from, the coding sequence. Using the Curtis/Weissmann technique in conjunction with a recombinant, true processing kinetics could therefore be studied for the first time leading to greater understanding of control of eukaryotic transcription.

with a mass of about $2.5 \text{ GeV}/c^2$. This is interesting, because it means that we are apparently observing the spectrum of excited states of the charmed particles, both for the baryons in this experiment, and for the mesons in the e^+e^- experiment. Theorists can therefore begin to test quite detailed dynamical models which may shed a great deal of light on the question of quark confinement and the fundamental nature of the strong interaction.

But the most direct probable observation of charm so far comes as a single event reported this week. (Burhop *et al.*, *Phys. Lett.*, in the press). Physicists from ten laboratories in Britain, the USA, Belgium, Switzerland, France and Italy exposed a stack of nuclear emulsion to the neutrino beam at Fermilab. They used spark chambers and counters to identify the possible neutrino events, and followed the particles seen in the spark chambers back through the emulsion to find the primary interaction points. Nuclear emulsions form the tracks of silver grains along the paths of charged particles, and these tracks can be resolved with a precision of around one micrometre. This is much finer than any other particle detector, and it means that the tracks of very short-lived particles can be observed, not just the tracks of their decay products. The photograph is a mosaic of pictures taken through a microscope of the tracks in the interesting event. A number of heavy tracks, and some very light ones can be seen emerging from the neutrino interaction point A, near the left of the picture. One of the lighter tracks can be seen to travel about $180 \mu\text{m}$ and then split at B into three light tracks, with no sign of any heavy recoil. Although one event can never be uniquely identified with complete certainty, by far the most straightforward interpretation of this event is as the production and decay of a charmed particle. The lifetime predicted by the GIM model is a few times 10^{-13}s , which gives a typical decay length of about $100 \mu\text{m}$, very consistent with the event observed. Detailed measurements on the tracks in emulsion show that there must be a missing neutral particle at the decay point, and examination of the spark chamber pictures has shown convincing signs of a K^0 or Λ^0 decay, within the spark chamber array, pointing back very satisfactorily to the event.

This strange particle may well be the only missing neutral, in which case it is possible to estimate the mass of the particle which decayed. Such estimates require further assumptions about the charged tracks from the decay. Are they pions or kaons? Assuming they are pions, the mass of the decaying

Likely examples of charm

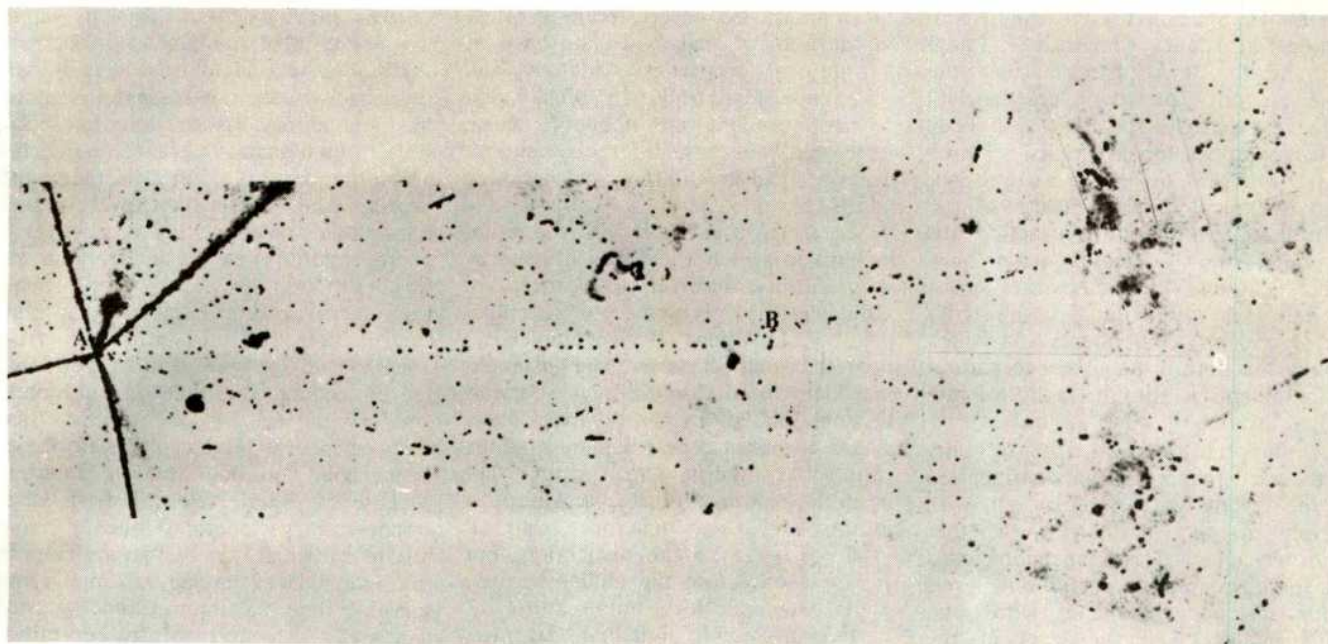
from David Miller

THERE is something very coy about charm. This new quantum number was first invoked to explain the absence of neutral-current processes in the weak decays of strange-particles (see for example, Glashow, Iliopoulos and Maiani, *Phys. Rev.*, **D1**, 1285; 1970; often known as "GIM"). A K^+ meson, for instance, will decay preferentially to a positive muon and a neutrino—a charged lepton system, but a K^0 produces the equivalent neutral lepton system, a positive and a negative muon, with an almost negligible probability. The first experimental evidence for the existence of charm was very indirect; the observation of the new J/ψ family of particles which are now thought to contain "hidden charm" in the form of a quark-antiquark pair. This year the e^+e^- colliding-beam machines have begun to show evidence for the production of pairs of overtly charmed mesons (see for example, Goldhaber *et al.*, *Phys. Rev. Lett.*, **37**, 255; 1976) and neutrino experiments at Fermilab near Chicago and at CERN at Geneva have continued to accumulate events with two leptons and a strange particle in

the final state, which are also usually regarded as examples of charmed particle production (see for example, Benvenuti *et al.*, *Phys. Rev. Lett.*, **34**, 419; 1975; Bleitschau *et al.*, *Phys. Lett.*, **60B**, 207; 1976). Two new results have now appeared to reinforce our belief in the quantum number.

A group from Columbia University, Hawaii, University of Illinois and Fermilab, working in a high energy photon beam at Fermilab, have observed the decay products of a long-lived antibaryon with a mass of $2.26 \text{ GeV}/c^2$. It decays into an antilambda hyperon (Knapp *et al.*, *Phys. Rev. Lett.*, **37**, 882; 1976) and three pions.

The GIM model predicted that the weak decays of charmed particles would produce strange particles, like the antilambda, as a direct consequence of the absence of strangeness-changing neutral currents. Not only does this experiment report the possible "charmed lambda" at $2.25 \text{ GeV}/c^2$, but they see strong indications that it may be produced by way of the decay of a heavier charmed baryon



particle is too low to correspond to the observed charmed mesons (with the neutral taken as a K^0) or the charmed baryons (with the neutral as a Λ^0). If two of them are kaons the mass estimates fit in quite well as either a charmed meson or a baryon. There is no requirement, in any known model, that three strange particles (a lambda and two kaons) should be produced in charmed decays, but there is no reason why it should not happen in perhaps 10 or 20% of events.

This event came from only fifteen neutrino interactions which have so far been identified in the emulsion. The collaboration is still searching for others, but a much larger exposure is planned next year at CERN, where the

new 400 GeV SPS accelerator will be used. Neutrino interactions in the emulsion will be found by projecting back tracks seen in a hydrogen bubble chamber, so the final state particles will be much more clearly identified than they are in the spark chambers. Two or three more events like this one will probably be sufficient to establish, at last, that it is charm that has been discovered. This would not only be satisfying as a vindication of the GIM model; the model is intimately linked with the attempt to unify the theories of the weak and the electromagnetic interaction, so the confirmation of charm will be a sign of real progress in this extremely important programme.

position of the peak response in the array of fibres leaving the cochlea, and intensity by the rate of discharge in the cochlear nerve. The problem arises with stimuli containing several frequencies, because discharge rate has been shown electrophysiologically to saturate at 50 dB, at which level different frequencies should (on position theory) become indistinguishable. But J. P. Wilson and J. Seelman (Keele University) have shown psychophysically that sound level has little effect on the ability of the ear to distinguish the constituents of different sounds (and besides, experience both inside and outside the laboratory amply demonstrates that speech is still intelligible at 70 dB).

Once beyond the auditory periphery, questions of auditory coding begin to overlap with those of visual coding, the central issue being whether single neurones which respond selectively to specific stimulus "features" are a plausible neurophysiological substrate for individual percepts. There have been claims, for instance, that some vertebrate neurones respond specifically to the mating calls of conspecifics.

Those claims, however, are subject to the objections that were raised in connection, for example, with visual "hand-detectors" in the infero-temporal cortex: the methods used simply could not identify such neurones, even if they existed. To resort to *reductio ad absurdum*, on the criteria applied to call-detectors many auditory cortical neurones are white-noise detectors.

More stringent criteria have since been applied by D. Ploog (Max-Planck-Institut) and his colleagues, using the natural calls of squirrel monkeys. Work based on the same principles but with

Sounds and signals

by Miranda Robertson

The Dahlem Conference on the Recognition of Complex Acoustic Signals was held in Berlin on September 27–October 1. A book based on the conference will be published in Spring 1977 and can be obtained from Dr Silke Bernhard, Dahlem Konferenzen, Delbrückstrasse 4c, D-1000 Berlin 33.

IN tackling the issue of how the physical properties of an auditory signal are analysed at various levels of the nervous system to produce a "percept", participants at the meeting were inevitably recapitulating for much of the time arguments that have preoccupied

visual and auditory neurophysiologists for many years and remain unresolved (for an example of the state of the question as visual neurophysiologists left it, see H. B. Barlow, *Perception*, 1, 371; 1972).

But although many of the general questions thus had a somewhat familiar air, some of the specific ones which were addressed exclusively to problems in audition presented a fresh challenge of both theoretical and, often, practical importance.

For example, neural coding is still not properly understood at the auditory periphery, as E. F. Evans (University of Keele) pointed out. It is generally accepted that frequency is coded by the

guinea-fowl was reported at the meeting by H. Scheich (Technische Hochschule Darmstadt), who with G. Langner and D. Bonke has identified the salient features (on behavioural criteria) of several species-specific calls. They tested neurones for selectivity in three ways: with variants of the natural call, with individual salient features of the natural call, and with synthetic calls compiled from those components. They concluded that "call-detectors" consist of neurones with combined selectivity for several call components and which would thus both respond best to natural ("broad-band") stimuli, and tolerate some variation in those stimuli.

An important preoccupation of many of the participants was the identification of the salient features of speech, with a view to the question of whether neurophysiological specialisations exist for speech perception at different levels—for instance, phoneme, word and sentence level. At the level of the phoneme, for example, very small variations in the physical signal can carry enormous linguistic weight. A. M. Liberman (Haskins Laboratories Inc.) and D. B. Pisoni (Indiana University) described work showing that the distinction between "slit" and "split" is made on the basis of the duration of the silence between the "s" sound and the onset of voicing with the "l". "Rabid" is distinguished from "rapid" in a similar way, although other cues (twelve of them) are available to help with that distinction.

At that level, little physiological specialisation seems to be necessary, since chinchillas (which happen to be reasonably sensitive to speech frequencies) do quite well on the rapid/rapid distinction (J. D. Miller, Central Institute for the Deaf, St Louis).

On the other hand, analyses of this kind may be crucial for the understanding of the aetiology of speech problems in children. P. J. Tallal (J. F. Kennedy Institute, Baltimore) reported that the cause of aphasia in some children had been identified as a specific inability to perceive rapidly changing speech sounds—a defect that can be found in adults with certain kinds of temporal lobe lesions.

But while the children can neither understand nor (consequently) produce speech, the lesioned adults can do both. This raises another important point about speech perception. That is that adult speech perception is determined almost as much by experience as it is by the physical speech signal. Pisoni and Liberman pointed out that, for example, coarticulation in normal speech obliterates important cues at the phoneme level, yet speech is still intelligible. That is because the listener already knows a great deal about the structure of language and

(usually) the domain of discourse, and understands the speech stream as much in the light of that as on the basis of its physical properties. Children lack that knowledge and are thus much more dependent on phonetic cues: hence, probably, the difference between Tallal's aphasic children and lesioned adults.

To return to parallels with the visual system, in which the focus of controversy has been how far early experience influences the development of receptive fields, P. D. Eimas (Brown University) suggested that there may be instances in which the auditory system, too, can become biased by early experience. There is evidence that Japanese adults cannot distinguish "ra" from "la" (really); yet Eimas finds that American infants can. Does this mean that all infants can make the distinction, but Japanese ones lose the ability because their language does not require it? Participants pointed out that that question could not be answered until it had been established that Japanese babies could make the relevant distinction, and that the field was fraught with methodological problems because of the nature of the tests on very young infants. A. J. Fourcin (University of London) added that it would be surprising if true, since the evidence says that perception of speech sounds is relatively poor in infants but improves up to the age of 13 or 14.

Plasticity in the auditory system was in fact a topic of extensive discussion, and this report no more does justice to it than it has been able to justice to many other lines of work drawn together by the organising committee under the chairmanship of T. H. Bullock (University of California, San Diego). For reams of fascinating information on echo-location by bats (G. Neuweiler, University of Frankfurt and N. Suga, Washington University), a cogent discussion on whether syntax and categorical coding exist in animal communication (P. R. Marler, S. M. Green, Rockefeller University), and the details of what machines can and cannot "understand" (chiefly M. Schroeder, University of Göttingen) and how to induce them to speak English without a heavy foreign accent—it will be necessary to read the book.

from E. F. Evans

How a scientific study of the biological mechanisms underlying the analysis of complex sounds can aid our understanding of disorders of hearing and language, was the subject of an additional session of the Dahlem Conference on the Recognition of Complex Acoustic Signals. This session devoted most of its time to considering the underlying nature of disorders of hearing and language before moving on to

consider implications for present and future methods of diagnosis and rehabilitation of the deaf.

Because we cannot yet identify all the stages of analysis of speech signals by the auditory system, it is not possible to understand the defective mechanisms responsible for disorders of hearing and language. However, the importance of processing at the peripheral auditory level, and lesions at this level, was stressed. Normal cochlear function is necessary for the correct detection, resolution (in frequency and possibly time), and intensity coding of simple and complex auditory signals, and lesions at this very peripheral level can therefore be responsible for poor speech analysis because of deterioration in frequency response, in peripheral frequency resolution (by which the ear separates out the individual frequency formants of speech), abnormal intensity coding and increased distortion. More centrally, disturbances in temporal analysis (particularly the processing of rapidly changing sounds, including formant transitions) have been shown to underlie speech processing deficits in aphasic children formerly labelled as "language impaired" (Tallal). In other words, the deficits lie in "lower level" acoustic signal processing rather than in "higher level" linguistic domains. Much more work, using the techniques of psychoacoustics and electrophysiology in the laboratory and in the clinic, is required to identify specific defects in speech processing.

It is clear that new strategies in the diagnosis of hearing and language disorders wait upon a more complete understanding. However, new tests are already indicated and are being evaluated. Routine threshold tone audiometry cannot, except in the extreme cases, lead to a prediction of the ability to process speech; on the other hand, the redundancy of the speech signal means that speech audiometry gives little information other than the degree of handicap. New tests are being devised to evaluate the functional state of specific mechanisms of speech analysis, using non-verbal speech-like signals. Thus, tests of peripheral frequency selectivity are being examined; other tests can be devised to evaluate both peripheral frequency selectivity and central pattern recognition (J. L. Goldstein, Tel Aviv University); and a battery of tests is in use to investigate systematically the normality of temporal and spectral analysing mechanisms in patients having impairment of language acquisition in spite of normal pure tone audiometric thresholds (Tallal).

As far as rehabilitation was concerned, great interest was shown in the recent attempts to preprocess speech

signals for patients with peripheral hearing losses, particularly those using filtering and frequency-specific compression, or dichotic presentation of electronically separated formants (W. A. Ainsworth, Keele University). Preliminary results were encouraging, but it is too early to predict the practical outcome for the deaf. Attempts to provide the profoundly deaf with speech information through visual and tactile inputs have proved disappointing, although it is possible to introduce prosodic information such as intonation (important for interpreting the meaning of speech signals) successfully through a multi-channel vibrator prosthesis (A. Risberg, Stockholm University). The restrictions on the rate of information processing presented by the relatively low temporal resolution of the skin and retina indicate that electrical stimulation of the auditory system, for example, by cochlear prostheses, may offer the most likely chance of useful rehabilitation of the profoundly deaf not helped by hearing aids. Present work with single electrode cochlear prostheses is encouraging, particularly for imparting prosodic information, such as the fundamental frequency of speech as indicated by A. J. Fourcin (University College, London). In this way, it ought also to be possible to signal specific speech features such as the "voiced-voiceless" distinction. Both of these should constitute an invaluable aid to lip-reading. However, the full range of speech features required for complete intelligibility can probably only be imparted by multi-electrode stimulation with adequate electrical isolation of the electrodes. This does not seem to be realisable in the foreseeable future.

Honey in the tropics

from a Correspondent

The First Conference on Apiculture in Tropical Climates, was held in London on October 18-20. It was arranged by the International Bee Research Association, in conjunction with the journal *World Crops*. The Conference ended by framing 23 resolutions for submission to international and governmental agencies. A copy is available from the International Bee Research Association (Hill House, Gerrards Cross, Bucks SL9 0NR), together with details of the published proceedings of the Conference. A Second Conference on Apiculture in Tropical Climates is proposed for 1979 or earlier, if possible in the tropics.

The programme at this first conference ranged over topics as diverse as the

chemical nature of bee sex pheromones and the state of development of bee-keeping in Ethiopia. Tropical countries that want to increase their honey production are understandably tempted to import exotic races, strains, subspecies or species of honeybee that reputedly store honey faster and in larger amounts, but several speakers emphasised the difficulties, and indeed the dangers, of doing this. The aftermath of the 1956 importation of a few queens of the tropical African honeybee *Apis mellifera adansonii* into South America is common knowledge.

The European honeybee *Apis mellifera*, an excellent honey producer in many temperate parts of the world, has now been imported into various countries of tropical Asia where the native *Apis cerana* is not very productive. Some of the importations have been inexplicably disappointing. Soekiman Atmosoedaryo reported on a recent introduction of *Apis mellifera* into Indonesia by the Forest State Corporation, of which he is Director. Colonies had dwindled away and died, in a way that was only too familiar to other Conference delegates. Possible reasons include the lack of adaptation of *Apis mellifera* to the many natural enemies of honeybees in the tropics—including other insects, and birds and mites. Also this temperate-zone bee follows a diurnal foraging pattern that is ill adapted to tropical plants presenting their nectar and pollen only in early morning and late afternoon.

N. Koeniger (University of Frankfurt, Germany) reported research that throws light on another difficulty experienced by imported species—that of mating. Queens and drones of any *Apis* species fly during a specific period of the day. If one *Apis* species is introduced into the territory of another, whose queens and drones fly during a period overlapping that of the imported species, the imported queens are unlikely to mate. Local queens, which are far more numerous, produce the same sex attractant as imported queens, 9-oxodecenoic acid, so drones of both species chase them, but only the local drones can mate with them. Most of the drones chasing an imported queen will also be of the local species; they cannot mate with her even if they locate her, but they keep away imported drones which could mate.

Apis mellifera and *Apis cerana* are not known to coexist in nature; if they do so, it would seem likely that their mating flight periods have become differentiated, as Koeniger found with the three *Apis* species present in Sri Lanka.

The management of bees for honey and wax production poses very



A hundred years ago

The directors of the Swedish Government railways have turned their special attention to the frequent occurrence of colour-blindness amongst their engine-drivers and other officials. Prof. Holmgren has lately examined the whole staff of the Upsala-Gefle Railway, and amongst the 266 persons examined has found no less than eighteen who suffered from this defect, and who therefore were utterly useless and unfit for railway service.

A very fine new university building has been erected at Kiel, one marked peculiarity of which is that it has no "carcer", or prison, which hitherto, it seems, has been an invariable appendage to German universities.

From *Nature*, 15, November 30, 110; 1876.

different problems in tropical and in temperate climates. Most of the early studies on bees were made in temperate regions, and perhaps for this reason, bee behaviour there has often been regarded as normal, and behaviour in the tropics as aberrant, yet it may well be the other way round. When the food resources of an area fail, tropical honeybees commonly migrate to an area where forage exists, as entire colonies, leaving their established nests (T. M. Chandler, International Development Research Centre, Nairobi). As a means of survival this migration is an alternative to the massive food hoarding of honeybees which have to survive a winter dearth period.

Jim Nightingale (Sasumua Estates, Njoro, Kenya) has lived with bees in Kenya for 54 years, and he was able to describe bees' migration routes in earlier days when human use of land was sparse and primitive: to-and-fro movements between the highlands and the floor of the Rift Valley—between the Aberdare Mountains and Naivasha, between the Mau forests and the Valley, and between the Kipsigis areas and down towards Lake Victoria. The human population has now upset age-old patterns in Kenya, but Ethiopia is not yet changed. Mammo Gebreyesus (PO Box 30407, Addis Ababa) maintains apiaries in the highlands, and in the Rift Valley 55 km to the east and 1,500 m lower down. He achieves continuous honey production by moving his hives between the two apiaries at the migration periods, so that the colonies do not leave them.

Mammo Gebreyesus is one of Ethiopia's very few modern beekeepers:

of around one million beekeepers only thirty use modern hives. The fact that the country has three million primitive hives exemplifies the scope for modernisation of beekeeping in the tropics. But, in addition, many areas in tropical Africa, Asia and America are grossly underpopulated with honeybees, and nectar and pollen resources go to waste.

After the sessions on bees, bee management and handling bee products (especially honey and beeswax), came the final session on apicultural development programmes, and this could usefully have lasted two days instead of four hours. It was the first meeting between representatives of donor agencies (from four continents), specialists in charge of their beekeeping programmes, and counterpart specialists from developing countries. At least 53 beekeeping development programmes have been or are now in operation. Tecwyn Jones (Centre for Overseas Pest Research, Ministry of Overseas Development, UK), who opened the Conference, stressed the advantages of beekeeping as a family-level exercise for the peasant farmer. It requires little financial input, and only a few square metres of land; it can be undertaken by people of almost any age, and the commitment can be variable, from a minimal spare-time one to full-time professional occupation. Even spare-time, it may well provide a peasant farmer with a much larger cash income than his usual full-time farming gives him. And it is environmentally sound; nectar and pollen are rarely utilised to the full unless there are social bees to take them, and beekeeping does not compete with other agricultural activities. Indeed it can greatly enrich them, through the bees' pollination of crops. □

Boats from the past

A Symposium on Boat Archaeology was held at the National Maritime Museum on September 20-24, 1976.

from Lynette Hamblin

BOATS and ships are among the largest and most complex movable structures attempted by man throughout his history, and as such offer much insight into his technical capability and organisation throughout the ages. Boat archaeology, however, is still in its adolescence and the symposium brought together nearly 100 international experts to discuss a number of the basic disciplines relevant to this specialised branch of archaeology. Techniques for recording finds and

presenting information, the problems of building boat replicas, hypothetical reconstructions of boat finds and ancient boatbuilding methods were all covered.

In-depth field recording is essential in any archaeological excavation, particularly in the case of wreck sites underwater, where material which has been perfectly preserved is prone to rapid destruction once on dry land. Whereas objects can be lifted and preserved without overwhelming difficulty, the timber of a ship presents a different problem. Lifting any boat can overstrain the conservation resources of a national museum and, unless special units can be set up to deal with specific craft, it is impracticable to haul hundreds of feet of waterlogged timber out of their protecting environment into an atmosphere where they will quickly twist, warp and ultimately disintegrate if not treated. The great ship of the Egyptian Pharaoh Cheops was preserved for 4,500 years in a bone dry, hermetically sealed pit; in the mere six years since it has been excavated and reconstructed condensation and lack of climatic stability has set up drastic deterioration. The air conditioning in the glass-walled museum that houses it is inadequate and the stress on the rebuilt timbers is proving critical.

As naval architect J. F. Coates pointed out boat reconstructions present many problems. Very often a boat find consists of only the bottom of the hull and one, if not both, ends may be missing too.

The Bronze Age boats found at North Ferriby present reconstructors with many puzzles. Nearly all the main pieces forming the bottom, and a bit of one end of a lower side strake were found; these were massive pieces of hewn oak sewn together with yew twig stitches. Coates described three possible reconstructions based on this material. The first was an effort to design a boat with the smallest amount of extra material in it. Such a boat could ferry ten people and eight paddlers across the Humber. The second reconstruction was a more ambitious and higher quality craft which could carry a larger load. However, strains increase with load and weight so design number two may be asking too much of its moss caulking—it would be necessary to find out how badly the boats leaked and this calls for a replica. His third reconstruction moved away from the actual remains. He combined the very heavy oak pieces forming the lower shell with a much lighter skin construction for the upper parts. The adze marks on the North Ferriby boats are of the maximum size possible with the available tools in-

dicating that these boats could have been the Concordes of their time—"in proportion to the Gross Tribal Product they could have been as expensive but arguably more useful".

However, no matter how well a reconstruction is worked out by theory and applied knowledge no naval architect would be too confident of a boat's practical performance unless a replica had actually been made and tested at sea. The National Maritime Museum has pioneered this form of experimental archaeology in its practical research with a replica of the Gokstad *Faering*. Line drawing and a construction plan of the Oslo *faering* were published by A. E. Christensen in 1966 and these were of use in building the replica. However, it was only once the *faering* had been rebuilt and was undergoing sea trials that it was realised why the Oslo *faering* had such large holes in the forward *stammering*—the holes in the NMM *faering* were too small for a man's hand making it difficult to haul the boat up the beach.

Experimental archaeology is concerned with the processes and production methods used in the past, and with the function of the artefact. The reconstruction of an ocean-going skin boat, the Brendan exemplifies the many factors that need to be considered. This type of boat may have been used in the west of Ireland between the 5th and 9th centuries AD. Evidence for their design was gathered from references to skin boats in contemporary texts, ethnographic evidence of the skin boats descendants, surviving examples of leather boats (all river craft), materials research as well as research into the contemporary techniques available for leather and timber working.

With the help of the British Leather Manufacturers' Research Association a test programme was conducted into the properties of various leathers. It was found that by far the most suitable leather for the hull covering was the mediaeval type of leather, tanned in oak bark. A naval architect produced the plans for the replica and shipwrights were asked to fair up a wooden frame to these drawings. Only timber available in Ireland in the early Christian era was used. oak for the gunwales and thwarts, ash for the frames and stringers. The frame was lashed together using alum-tanned leather—a technique known in Roman times—and the entire structure was coated with wool grease to preserve the ash frame and protect the leather. Forty-two ox hides were needed to cover the boat, stitched together with hand-rolled flax. The Brendan has now successfully sailed to Greenland where it will have to spend the winter before completing the trip to North America.

articles

Implications of pollen assemblage from the Koobi Fora Formation, East Rudolf, Kenya

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A pollen spectrum obtained from the Ileret Member of the Koobi Fora Formation indicates that vegetation patterns differed significantly from those prevailing east of Lake Rudolf today. The pollen-bearing sediment, which is interstratified with Plio-Pleistocene hominid fossils, shows somewhat more humid conditions.

PLIO-PLEISTOCENE lacustrine and fluvial strata exposed along the eastern side of Lake Turkana (Rudolf), Kenya are notable for their rich assemblages of fossil vertebrates¹, hominid remains² and early artefacts³. The geology and stratigraphy have been described by various authors who have worked in this area since 1969 (see refs 4-6).

Preliminary samplings for pollen analysis were taken at different localities where outcrops of the Koobi Fora Formation are exposed. Of the 27 samples, collected and examined, only one yielded pollen in sufficient quantity to justify study and interpretation. The pollen assemblage, Koobi Fora Formation Pollen Spectrum No. 1 (KFFP 1), was extracted from sandstone 3 m above the hominid site KNMER 1592 at Ileret, area 12 (Fig. 1). It comes from a stratigraphic level situated between the KBS Tuff and the Lower/Middle tuff complex. Two different sets of dates have been proposed for the KBS Tuff, one⁷ assigning it an age of 2.6 ± 0.26 Myr and the other assigning⁸ an age of 1.6 ± 0.05 Myr to the Upper KBS Tuff and 1.82 ± 0.04 Myr to the lower tuff.

The Ileret Lower/Middle Tuff complex has been dated⁹ at 1.48 ± 0.17 Myr. The pollen sample comes from just above the level which is interpreted equivalent in time to the post erosion surface (I. Findlater, personal communication) at an horizon that shows reverse polarity in other sections. From this it follows that if the first hypothesis is correct, the stratigraphical level from which the pollen assemblage was extracted would be dated between 1.8 and 2.4 Myr. For the time being it seems reasonable to treat the age of the pollen assemblage as being $\sim 1.5-1.6$ Myr.

Pollen extraction was undertaken on a 100-g sample of sediment after a positive result had been obtained with a 20-g sample. The chemical process involves first an attack with a 10% solution of HCl, dissolving the silicates with a 70% solution of HF, and subsequent density separation of the organic matter using 'Liqueur de Thoulet'. All the residue has been observed. The total count of pollen (696) represents the absolute quantity recorded in 100 g of sediment. No heavily corroded pollen grains were noticed.

Palynological results

Details of the pollen assemblage are given in Table 1. Identifications of pollen grains are based on a reference

slide collection which contains $\sim 4,000$ East African species. Morphological studies of > 350 species from the Omo area including light and scanning electron microscopic observations were also used. As can be seen in Table 1, some taxa have been identified at the family level, others at the generic or specific level.

Fig. 1 Map of the East Rudolph region, showing the sampling areas.

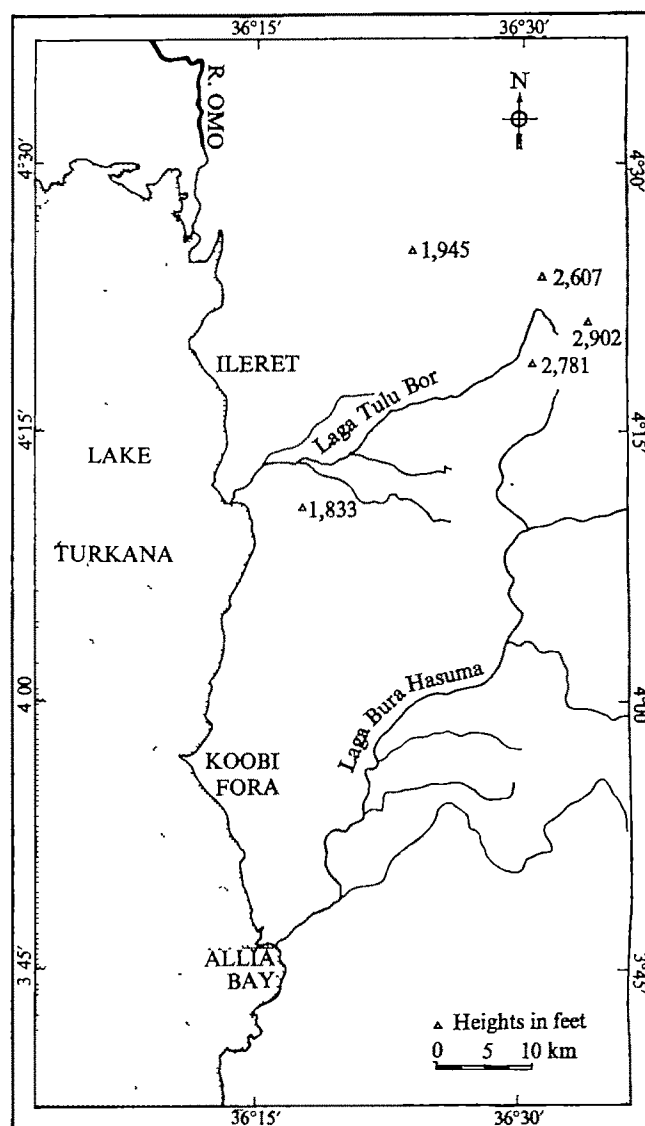


Table 1 Fossil pollen spectrum of a sample from the Koobi Fora Formation (Illet, East Rudolf, Kenya), as percentage of the total

(1) Taxa not represented in the East Rudolf area*		No.	%
A Taxa present in the Ethiopian Highlands			
Ericaceae		2	0.2
Podocarpaceae	<i>Podocarpus</i>	19	2.7
Cupressaceae	<i>Juniperus</i>	26	3.7
Oleaceae	<i>Olea</i> cf. <i>O. hochstetteri</i>	1	0.1
Rosaceae	<i>Hagenia abyssinica</i>	4	0.5
Sapindaceae	<i>Dodonaea viscosa</i>	8	1.1
Euphorbiaceae	cf. <i>Croton</i>	1	0.1
Myricaceae	<i>Myrica</i>	1	0.1
Sp. A cf.	<i>Bosqueia</i>	16	2.2
Sp. B cf.	<i>Meliaceae</i>	3	0.4
Compositae liguliflorae		1	0.1
Compositae	<i>Artemisia</i>	1	0.1
Acanthaceae	<i>Mimulopsis</i>	1	0.1
Plantaginaceae	<i>Plantago</i>	4	0.5
Urticaceae	<i>Droguetia</i>	4	0.5
Caryophyllaceae		3	0.4
	Subtotal:	95	13.6%
B Taxa present in riverine vegetation near the Omo river			
Anacardiaceae	<i>Rhus</i> sp.	2	0.2
Moraceae	<i>Ficus</i>	1	0.1
Euphorbiaceae	<i>Ricinus communis</i>	1	0.1
Celastraceae	<i>Maytenus</i>	3	0.4
Combretaceae	<i>Combretum</i>	2	0.2
Palmae	<i>Hyphaene</i>	1	0.1
Amaranthaceae	<i>Celosia argentea</i>	2	0.2
Amaranthaceae	<i>Celosia</i> cf. <i>trigyna</i>	3	0.4
Acanthaceae	<i>Justicia anselliana</i>	3	0.4
	Subtotal :	18	2.5%
	A+B =		16.2%
(2) Taxa present in the East Rudolf area			
Capparaceae	<i>Cadaba</i>	5	0.7
Capparaceae	<i>Boscia</i>	1	0.1
Mimosaceae	<i>Acacia</i> (2 pollen types)	18	2.5
Burseraceae	<i>Commiphora</i>	5	0.7
Salvadoraceae		37	5.3
Amaranthaceae	<i>Sericocomopsis</i>	1	0.1
Tiliaceae	cf. <i>Grewia</i>	1	0.1
Acanthaceae	<i>Justicia</i> cf. <i>odora</i>	1	0.1
Compositae	cf. <i>Pluchea</i>	1	0.1
Chenopodiaceae		30	4.3
Amaranthaceae	<i>Digera</i>	3	0.4
Euphorbiaceae	<i>Euphorbia</i>	35	5.0
Solanaceae	<i>Solanum</i>	1	0.1
Nyctaginaceae	<i>Commicarpus</i>	1	0.1
Acanthaceae	<i>Blepharis</i>	1	0.1
Liliaceae		3	0.4
	Subtotal:	144	20.6%
(3) Ubiquitous taxa			
Compositae tubuliflorae		14	2.0
Acanthaceae		2	0.2
Gramineae		303	43.5
Typhaceae	<i>Typha</i>	17	2.4
Cyperaceae		75	10.7
	Subtotal:	411	59.6%
(4) Pteridophytes		15	2.1%
(5) Unknown		13	1.8%
Total =		696	
Spores Anthocerotales: 12			

*Not recorded in the checklists established by J. B. Gillett, East African Herbarium (1971, 1974).

Taxonomic identifications

Taxa identified as Gramineae, Cyperaceae, Compositae, Liliaceae, Caryophyllaceae belong to botanical families in which pollen grains are very homogeneous. Pollens of grasses are porate, with one pore and a smooth exine. They offer very few characteristics which could be used for generic diagnosis. In the fossil pollen assemblage, grasses are very abundant, representing 43.5% of the total count. This percentage includes grasses growing near the lake shores or river banks as well as grasses in the savanna which cannot be distinguished on the basis of diameter measurements. Other taxa identified as Ericaceae, Acanthaceae, Liliaceae represent fossil pollen grains in which some details are not preserved, preventing identification beyond the family level.

Generic and specific identifications of the Amaranth pollen grains are based on recent work by Rioulet and Bonnefille⁹. Of the genus *Olea*, six species are known in the East African flora. Two separate pollen types can be distinguished, the type *O. africana* (= *O. chrysophylla*) in which the exine shows a reticulum with large lumina and the type *O. hochstetteri* which has smaller lumina.

In Table 1, specific identifications are given for pollens recognisable by morphological details, which belong to contemporary African genera only known through a single species. These are *Dodonaea viscosa*, *Hagenia abyssinica* and *Ricinus communis*. Within other genera such as *Justicia*, certain species show strong differences. Pollen grains of *Justicia anselliana* are small (~20 µm) and diporate. They have no furrows visible and the exine is of about the

same thickness at the pore and at the equator. *Justicia odora* and *Justicia fischeri* pollen grains are dicolporate with a long visible furrow, their size is $\sim 35 \mu\text{m}$. They cannot be distinguished from each other, and so the fossil has been designated as *Justicia* cf. *odora* type. But this pollen is very different from *J. flava* or *J. striata* which show a very strong thickening of the exine at the equator.

Pollen grains classified under species A are represented by periporate grains of $\sim 20 \mu\text{m}$ with 12 to 15 pores. The pores are well-delimited with an operculum. On the optical sections very fine columellae are barely seen. Such pollen exist among Plantaginaceae (*Plantago*) and Moraceae (*Bosqueia*). Considering the exine structure and the pores, the fossil pollen grains closely resemble those of *Bosqueia phoberos* (= *B. angolensis*) a common tree of the most humid high forest of western Ethiopia and of the rain forest in the Congo. The regular undulations with the verrucate or microechinate sculpturing which characterise the exine of pollen grains in the genus *Plantago* have not been clearly observed on the fossil pollen. In the present East African species of *Plantago*, the pore outline is not well delimited and the pore membrane is covered with granules. No distinct operculum is visible, except in *P. lanceolata*. But in this species, the exine is echinate. The fossil pollen grains cannot be attributed to the genus *Plantago*. They are considered to be close to *Bosqueia*. The possibility that they could belong to an extinct species cannot, however, be excluded.

Pollen grains of species B are tricolporate with granulate colpi membranes, a smooth exine surface and no distinct columella. Similar pollen exists within the 'Meliaceae' family. The fossils, however, are not identical to the living African species of Meliaceae which we have observed.

Pollen grains similar to species A or B have never been recovered from the modern pollen rain content of the East Rudolf or Omo areas. Whatever their precise identification, they have to be included in group A of Table 1.

Comments on the fossil pollen assemblage

Within the fossil pollen assemblage the different taxa have been arranged in five groups (Table 1).

The first group consists of taxa which are not represented today in the vegetation of the East Rudolf area according to the lists established on the basis of the collections of plants made by many authors. This first group has been divided into two parts, A and B. A includes taxa from montane forests of the Ethiopian highlands and B taxa from riverine plant communities. In the second group are

considered taxa from the surrounding semi-desert steppes of the Lake Turkana area. Within a third group are assembled ubiquitous taxa among which grasses are particularly abundant.

As we cannot distinguish the different genera of grasses which belong to distinct communities, we must consider them as a whole. *Typha* is usually found near fresh water but Cyperaceae (sedges), which can be very abundant near saline or alkaline lakes, do exist within savannas among other herbaceous plants. Compositae of the tubuliflorae type as well as Acanthaceae have been classified as ubiquitous taxa because of the imprecise nature of their identification. The fourth and fifth groups are respectively represented by the pteridophyte spores and the unidentifiable pollen.

The survey of the fossil pollen assemblage (Table 1) shows that among the 47 pollen taxa recorded (pteridophytes included), more than half of them, that is 25 (group 1), are not present in the contemporary East Rudolf vegetation. Nine of them may occur in riverine communities along the Omo river and in the delta, north of Lake Turkana. The other 16 exist, now, in the high forests of south-western Ethiopia, in the Gemu-Gofa and Sidamo provinces, 150 to 200 km east or north of Meret. The great number of taxa which are not represented in the vegetation of the East Rudolf area today seem to be a remarkable feature of this fossil pollen assemblage.

Comparison with palynological results from modern sediments

Samples of recent sediments were collected as a part of A. K. Behrensmeyer's research into taphonomy. They were taken from lake margin environments at Alia Bay and from modern fluvial ones at the Laga Bura Hasuma and Tulu Bor, both of which are seasonal water courses (Fig. 1).

Table 2 summarises the percentages of pollen of the previously defined groups in these samples, detailed counts will be found in another paper¹¹. For the palynological data obtained from recent sediments, pollens which belong to taxa from montane and forest communities are considered as allochthonous, that is, transported from the source to the place of deposition. Allochthonous pollen give information on the vegetation of the whole basin.

Implications for regional vegetation patterns

In all the studied samples from recent sediments, the percentages of allochthonous pollen are very small (0.04–2% of

Table 2 Distributions of various groups of pollen taxa in fossil against modern surface sediments of Lake Turkana area, Kenya

	East Rudolf	Highlands			Riverine			Savanna			Ubiquitous			Pteridophytes			Total count
		No.	(a)*	(b)	No.	(a)	(b)	No.	(a)	(b)	No.	(a)	(b)	(a)	(b)		
Fossil	Meret KFFPI	16	13.6	24.2	9	2.5	4.5	17	20.6	36.6	5	59.6	27.4	2.1	3.8		696
	Alia bay																
	— AB Ia/B ₁	1	0.5	0.9	—	—	—	7	49.9	95.3	4	49.6	3.7	—	—		976
	— AB Ia/B	1	0.3	0.9	—	—	—	4	31.6	91.3	4	68.0	7.5	0.1	0.3		959
	— AB Ia/A	1	0.2	3.0 ⁽¹⁾	—	—	—	2	6.0	86.4 ⁽¹⁾	4	93.8	10.6 ⁽¹⁾	0.	—		957
	— AB Ic/A _{90b}	2	0.5	0.9	—	—	—	7	50.8	97.6	3	48.3	0.8	0.1	0.2		967
Modern	Laga Bura Hasuma transect																
	— BH I/A ₁	1	0.5	1.9 ⁽³⁾	2	4.7	—	26	24.2	94.1	3	28.0	2.7	0.2	0.8		1,000
	— BH I/A ₂	2	0.04	1.2 ⁽³⁾	3	2.5	1.2	24	2.9	86.9	4	94.6	10.6	—	—		4,789
	— BH I/A ₁₁	4	1.5	4.3 ⁽³⁾	4	2.7	2.0 ⁽³⁾	27	29.6	85.8 ⁽³⁾	4	66.2	7.8 ⁽³⁾	—	—		1,000
	Laga Tulu Bor transect																
	— A ₈	3	0.5	0.5	—	—	—	19	85.4	95.3	2	13.6	3.6	0.5	0.5		617
	— A ₄₋₅	3	2.0	3.7	1	0.3	0.6	26	46.5	86.8	4	51.1	8.6	—	—		608
	— A ₁₁	4	1.2	4.8	1	0.1	0.4	18	20.5	81.9	4	77.8	11.6	0.3	1.2		993
	— A ₁₇	2	0.7	2.0	1	0.2	0.5	17	31.7	91.2	4	67.2	5.6	0.2	0.5		558

Modern data are from ref. 11. The localities are shown in Fig. 1.

*% calculations: (a) total count; (b) total count excluding Gramineae and over-represented species (1) Cyperaceae, (2) *Combretum*, (3) *Combretum* + *Acalypha*.

Table 3 Comparative pollen taxonomic diversity (number of taxa) in Plio-Pleistocene against modern surface sediments from Omo and East Rudolf

Age	Samples	Highlands	Riverine	Savanna	Ubiquitous	Total
Modern	M AB	1.2	0	5	3.7	18
Fossil	M BH+TB	2.7	1.7	22.4	3.6	68
1.6-1.5 Myr	KFFP1	16	9	16	5	46
2.2-2.15 Myr	Omo E-4	5	1	10	3	19
2.45-2.4 Myr	Omo C-9	11	6	18	4	39
2.45-2.4 Myr	Omo C-7/C-8	10	6	23	3	42

M = mean of results from Table 2. C-8, C-9, E-4 are stratigraphic units from the Shungura Formation, Lower Omo Valley, Ethiopia.

the total count, or 0.5-4.8% of the total from which grasses are excluded). There are slight differences between the percentages of lacustrine and fluvial allochthonous taxa observed in the various samples. (Table 2).

By contrast the number of riverine and highland taxa is much more important in the fossil assemblage than in any recent samples (Table 3). Altogether the percentages of those taxa represent 16.2% of the total pollen (Table 1) or 28.7% of the total pollen after Gramineae are excluded (Table 4). Such large percentages of highland taxa have been recorded in a pollen spectrum of recent Omo river deposits only from a site 50 km North of the Omo delta and 150 km north of Ileret (Table 4). In this recent assemblage, allochthonous pollen from the Ethiopian highlands is brought down streams.

Considering the fossil data, there seem to be three principle lines of explanation for the higher percentages of allochthonous pollen compared with recent spectra: first, the allochthonous pollen may formerly have been introduced from the north-east by a large river which has now ceased to flow; second, the incidence of allochthonous pollen in recent sediments may have been artificially depressed by recent deforestation caused by human interference, and third, at the time of the deposition of the fossil sample, different environmental conditions may have permitted the growth of montane forest species closer to Ileret than today.

The first seems unlikely through a detailed reconstruction of the palaeogeography¹¹. Also bones and hominid remains, particularly abundant in area 12, have not been subjected to noticeable reworking. Modern sediments from the delta margin transect of Alia Bay are considered by Behrensmeyer as the closest model for deposition of the Lower Pleistocene sediments at Ileret. On the basis of the geological data, stream transport of pollen seems an unlikely explanation of the high value of mountain taxa in the fossil pollen assemblage. Furthermore, if the fossil pollen assemblage at East Rudolf was from a fluvial environment, it should also show a higher concentration of riverine taxa than are in fact recorded (compare data for recent sediments of the Omo River, Table 4). No pollen of *Zyzygium*, *Celtis* or *Ziziphus*, all common trees of riverine plant communities, have been recorded in this sample. Even if a part of the allochthonous fossil pollen was introduced by fluvial transport, it should be noted that the modern rivers on the eastern side of Lake Turkana do not transport comparable pollens. Also, the

whole picture given by the pollen spectrum is consistent with deposition in a situation between the distributary channels of a delta.

For the second possible explanation, it is apparent that if the differences between recent and fossil assemblages are to be attributed to intensified human activity, then all early fossil assemblages from Omo and East Rudolf should differ from the modern ones in a similar manner. Tables 3 and 4 demonstrate the contrary. Strong fluctuations in the amounts of montane forest pollen have already been noticed in Plio-Pleistocene sediments of the Shungura Formation in the lower Omo Valley¹².

Explanations of the contrast between the fossil and recent spectra which depend purely on differences of environment of deposition seem highly unlikely in view of the recent demonstration that these do not greatly influence the relative quantity of allochthonous pollen¹³. I therefore favour the third explanation, namely that the fossil sample reflects climatic and ecological environmental conditions differing significantly from those of the present day. At Ileret, 1.5 Myr ago, climatic conditions must have been cooler and more humid than today, and more favourable to extensive forests.

Implications for local vegetation patterns

In the fossil pollen assemblage from Ileret (Table 1) group 2, which contains the taxa recorded today in the East Rudolf vegetation, shows a great abundance of *Acacia*+*Capparaeae*+*Commiphora* and *Salvadoraceae*, all common trees of the dry woodland. Among the herbaceous taxa, *Chenopodiaceae*+*Amaranthaceae* are the most abundant, with the *Euphorbiaceae*. No interpretation can be made of the *Euphorbia* pollen since ~ 100 species with different ecologies are found in East Africa. It is not the *Euphorbia grandicornis* commonly found in this subdesert area.

Chenopodiaceae and Amaranthaceae

Among the *Chenopodiaceae* it was not possible to increase the precision of pollen identification to the generic level. The fossil pollens are very similar to pollens of *Suaeda* or *Fadenia*, very abundant today in the vegetation surrounding Lake Turkana. Altogether *Chenopodiaceae* + *Gramineae* + *Amaranthaceae* + *Cyperaceae* represent ~ 60% of the fossil pollen count. The recent lacustrine samples from East Rudolf are extremely consistent with high values

Table 4 Comparative pollen composition (as percentages) of Plio-Pleistocene deposits against modern surface sediments from Omo and East Rudolf

		Highlands	Riverine	Savanna	Ubiquitous	Pteridophyte	Total pollen count
Modern	Omo River	29.5	20.3	14.2	24.7	5.8	1,000
	M AB	1.4	—	92.6	5.6	0.1	3,859
	M BH+TB	2.6	0.7	88.8	7.2	0.4	9,565
Fossil	KFFP 1	24.2	4.5	36.6	27.4	3.8	696
	Omo E-4	6.4	0.3	84.4	5.7	1.0	1,062
	Omo C-9	23.6	11.8	12.8	49.6	0.7	693
	Omo C-7/C-8	38.8	6.0	23.0	24.9	1.0	1,072

% expressed as percentages of total count excluding Gramineae and over-represented species.

of Chenopodiaceae associated with Cyperaceae and Gramineae (97–99%) while the fluviatile series are more variable (62–96%).

Pollen analyses of surface sediment samples have shown high percentages of Chenopodiaceae in the subdesert and desert areas of the Sahara^{13,14}, in the arid zones of the Middle East, in Iran¹⁵, and also in the southwestern USA (such as Arizona¹⁶) as well as in dry continental areas of the USSR¹⁷. Chenopodiaceae are numerous in places with high salinity or alkaline soils.

On the basis of modern pollen rain studies, authors agree that high values of Chenopodiaceae pollen are obtained near places where the plants are found. Their density decreases rapidly with distance from the source. For this reason, high Chenopodiaceae frequencies in fossil data are basically the result of local conditions rather than regional ones. In this particular fossil assemblage, related to a delta margin environment, a percentage of Chenopodiaceae much lower than in the recent Alia Bay samples may indicate a lake less alkaline than the present one.

Woodland and bushland

Percentage calculations of the various savanna taxa included in the groups 2+3 (Table 1) of the fossil pollen assemblage are given in Table 5. They provide information on the composition of the local vegetation around Lake Turkana in the past. It should be noticed that the pollen composition of the fossil assemblage differs from all the studied modern spectra in containing a great quantity of *Acacia* and *Commiphora*. The ratio between arborescent and non-arborescent taxa calculated from Table 5 has about the same values as for recent pollen spectra from surface samples collected beneath tree cover near the Tulu Bor River, east of Lake Turkana¹⁹. We consider that the vegetation near the lake at the time of deposition of the sediment, was more wooded than today, and can be regarded as *Acacia-Commiphora* bushland. The great percentage of pollen of Salvadoraceae, in which the identification of *Salvadora* from *Dobera* is not possible, could reflect the mesic environment near a river delta.

Discussion

Because of the present lack of data on the pollen rain in tropical lowlands, great caution is needed in the palaeoclimatic interpretation of this pollen analysis. A pollen assemblage does not exactly reproduce the detailed composition of the regional vegetation, but it reflects the essential character of the flora which existed at the time of deposition. Such information on vegetation would not be available without palynological studies.

The composition of the plant community and the resultant pollen rain can be related by correlation factors to allow for the differential pollen production by wind-pollinated and insect-pollinated plants. For the moment no great precision is possible. But we have, in effect, taken those factors into account by comparing the fossil assemblage with assemblages of recent pollen deposited in the same area. More sophisticated treatment must await the compilation of much more comparative data on pollen production, absolute and relative dispersal in such tropical areas. In spite of the reservations just expressed, it remains true that plants constitute the most sensitive indicators of climatic, hydrological and pedological conditions of their habitat. The ecological requirements for certain species and vegetation communities are so precise that they can be used to characterise particular climatic regimes.

The fossil pollen assemblage from Ileret seems to permit a reasonably definite characterisation of the flora in the area, 1.5 Myr ago. The prominence of montane forest elements is particularly striking. Since it appears that this feature cannot be explained entirely by long distance wind or water transport, it seems clear that vegetation of highland forest type must have existed closer to the basin

Table 5 Distribution of savanna taxa (groups 2+3 of table 1) in the fossil pollen spectrum KFFPI

	No.	%*	%†
Trees and Shrubs			
<i>Acacia</i>	18	3.4	13.8
Capparaceae + <i>Grewia</i> + <i>Commiphora</i> + <i>Cordia</i>	12	2.3	9.2
<i>Salvadora</i>	37	7.0	28.5
Others	3	0.6	2.3
Herbaceous			
Acanthaceae	3	0.6	2.3
Euphorbiaceae	35	6.7	26.9
Amaranthaceae	3	0.6	2.3
Compositae	14	2.7	10.8
Others	5	0.9	3.8
Total	130		
Gramineae	303	57.7	
Cyperaceae + <i>Typha</i>	92	17.5	
Total	525		

* % Calculations with Chenopodiaceae excluded. Pollen sum 525.

† With Chenopodiaceae + Cyperaceae + *Typha* Gramineae excluded. Pollen sum 130.

margins. Further, because the contrast between recent pollen assemblages and the fossil one cannot have arisen simply from recent deforestation, the climate must surely have been somewhat cooler or wetter at the time of deposition of the sample. The vegetation in the vicinity of the sample site was dominated by Gramineae and Chenopodiaceae appropriate to the margins of a slightly saline or alkaline lake. On the other hand, the unusually good representation of *Acacia*, *Commiphora* and *Salvadora* implies a shrub and tree cover that was more dense than that prevalent in the basin today. Altogether the indications are of a climate that was neither excessively humid nor semi-arid.

The sample was taken from strata which contain early hominid fossils and archaeological remains of the Upper Member of the Koobi Fora Formation. The pollen spectrum of the Koobi Fora documents conditions existing at some point during the occupation of the area by evolving hominid populations. The palaeoenvironmental evidence provided by this sample, however, refers to a relatively short timespan during the deposition of the sediment containing the pollen spectrum. To assess the extent to which the pattern observed was stable, fluctuating or subject to persistent long term trends, we will need to procure spectra from other layers.

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Wind shear observations in thunderstorm density currents

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Acoustic sounder observations of thunderstorm density currents reveal a complicated but ordered internal structure. Fast-response anemometers on a 150-m tower reveal a succession of internal shear layers which occur following the leading portion or nose of the current. The measured wind shear revealed by the anemometers is found to be a function of the temperature differential between the ambient air and the interior of the gust, measured near the surface. An heuristic model is developed to explain this observation.

DENSITY currents flowing out from thunderstorms, sometimes referred to as gust fronts or microfronts, can constitute a hazard to the landing and takeoff of aircraft. The associated wind shear results in the aircraft experiencing rapid changes in air speed; vertical shears $> 0.1 \text{ s}^{-1}$ in the lowest 100 m have been shown to be hazardous to large, swept-wing, jet-powered aircraft in a study by Snyder¹. An improved knowledge of density current dynamics and structure is also important in understanding the interaction of the thunderstorm with its environment. Since the cold air outflows from storms are statically stable, but highly turbulent, such currents exhibit large acoustic index of refraction fluctuations, leading to strong acoustic echoes when the flows are probed with sound waves. Using an acoustic sounder as a guide in the interpretation of meteorological tower data we present new observations of the internal structure and shear in gust fronts, and find a strong correlation between the strength of the current and its temperature contrast with the ambient air.

Wind shear measurements

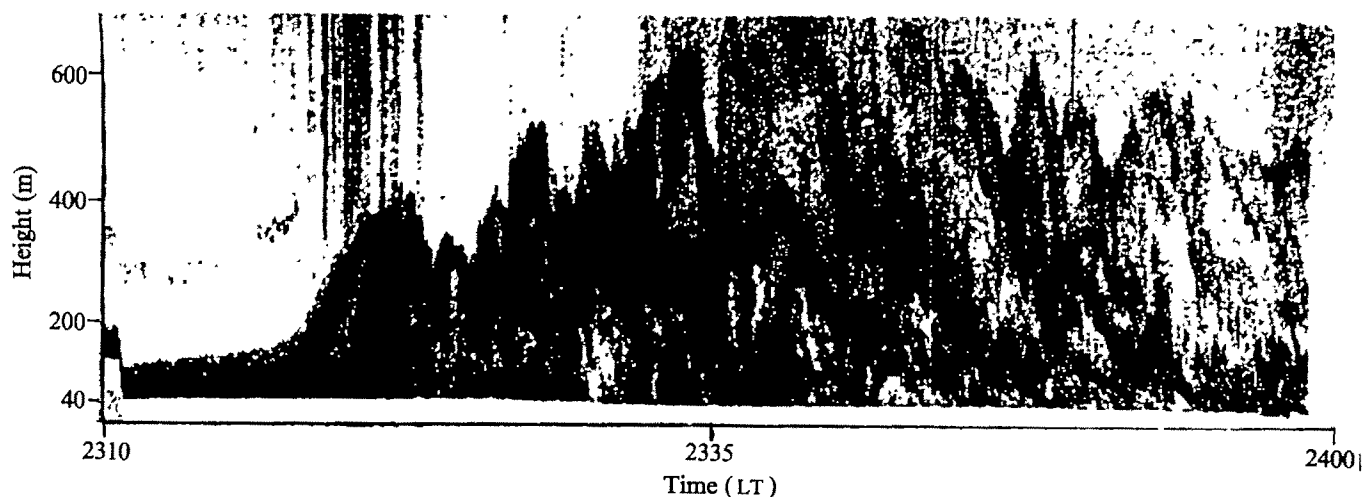
During field experiments in 1972 and 1974, five different thunderstorm density currents were observed at the National

Oceanic and Atmospheric Administration (NOAA), Haswell, Colorado site. The meteorological tower at Haswell, 150 m tall and located 300 m from an acoustic sounder, was instrumented at 30-m intervals with fast response wind and temperature sensors; data were recorded at 1.0-s intervals. In addition, heavy-duty wind sensors were located at 15 m and 150 m, and were able to withstand the high winds which in two cases destroyed the fast response instruments.

In the two more severe storms observed, wind-generated noise at the acoustic antenna masked the reception of echoes, but for the other cases, the internal shear structure was observed in detail with the sounder. The structure for the August 11, 1972 current is shown in Fig. 1. This outflow originated near a line of small thunderstorms which had levelled off at a peak altitude of 8.5 km above sea level, and were observed to dissipate 20 km NE of Haswell at 2300 by the National Weather Service weather radar in Limon, Colorado. When last observed the storms were moving 6 m s^{-1} from an azimuth of 330° . The maximum wind speeds within the current were 5 m s^{-1} from between 280° and 340° . This density current flow was opposed to an ambient flow of 10 m s^{-1} from 135° before the event, producing a vector change in wind speed, Δu of 15 m s^{-1} . A temperature drop of 2°C at 30 m occurred with the passage of the microfront or head of the current. Maximum shears at the lower edge and in the interior of the current reached values of $\partial u / \partial z = 0.13 \text{ s}^{-1}$ as measured by the tower bivanies; the strongest shear events correlated with temperature shifts measured on the tower and with the observation of scattering regions on the acoustic sounder facsimile record. This correlation is to be expected since theory predicts that the acoustic scattering is produced by small scale temperature fluctuations associated with turbulence in regions of temperature and wind gradients.

This prediction has been verified quantitatively by Neff² for a number of cases including elevated scattering layers. The

Fig. 1 Acoustic sounder facsimile record obtained on August 11, 1972 at Haswell, Colorado, of a density current originating from a line of weak storms more than 20 km away. Wind noise associated with the passage of the current nose led to the vertical noise-lines in the upper part of the record at 2320. The postulated shears and temperature gradients at the edge of the current, and in descending layers within the current, are revealed as darker regions on the facsimile record because of increased acoustic scattering cross sections.



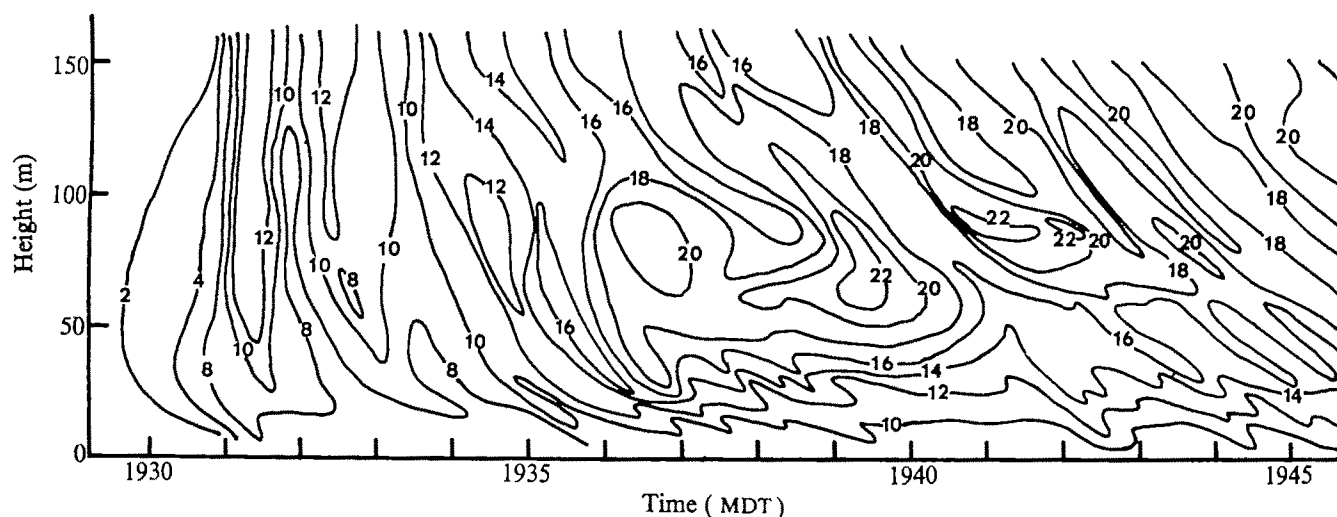


Fig. 2 Isotachs in m s^{-1} drawn against height and time as derived from wind measurements at six levels on the Haswell tower, 10-s averaged data for the density current of August 5, 1972. The resulting pattern of shear is consistent with the layered structure shown by acoustic sounder observations of much weaker currents as in Fig. 1.

thin, tilted echo layers, internal to the current but above the height of the tower, are thus presumably of such an origin. Although not verifiable from the present data, the local enhancement of the shear and consequent production of turbulence may arise from internal waves generated at the interface or large-scale overturning within the current.

Two more vigorous density currents from closer and larger thunderstorms passed the tower on August 2 and 5, 1972. In these cases, the wind noise was so great that acoustic records could not be obtained. By using the August 11 facsimile record as a guide in interpreting the wind data from the tower, however, a consistent set of isotachs could be plotted for the August 5 event following the form suggested by Fig. 1, with tilted shear layers gradually sloping or descending with time. These are shown in Fig. 2, plotted from data averaged over 10 s. One minute of data averaging proved to be too long to delineate the thin shear regions within the currents; therefore, internal shear layers were not revealed in the recent comprehensive thunderstorm outflow study by Goff⁴, who used 2-min wind averages.

From Fig. 2, it is immediately obvious that the vertical shear $\partial u / \partial z$ in the lower 50 m is consistently large and above the aircraft safety criterion set by Snyder¹. It must be realised that the spatial slope of the higher shear zones is greatly accentuated in Figs 1 and 2; if one assumes the zones are advected with the mean wind, the actual slopes for the shear

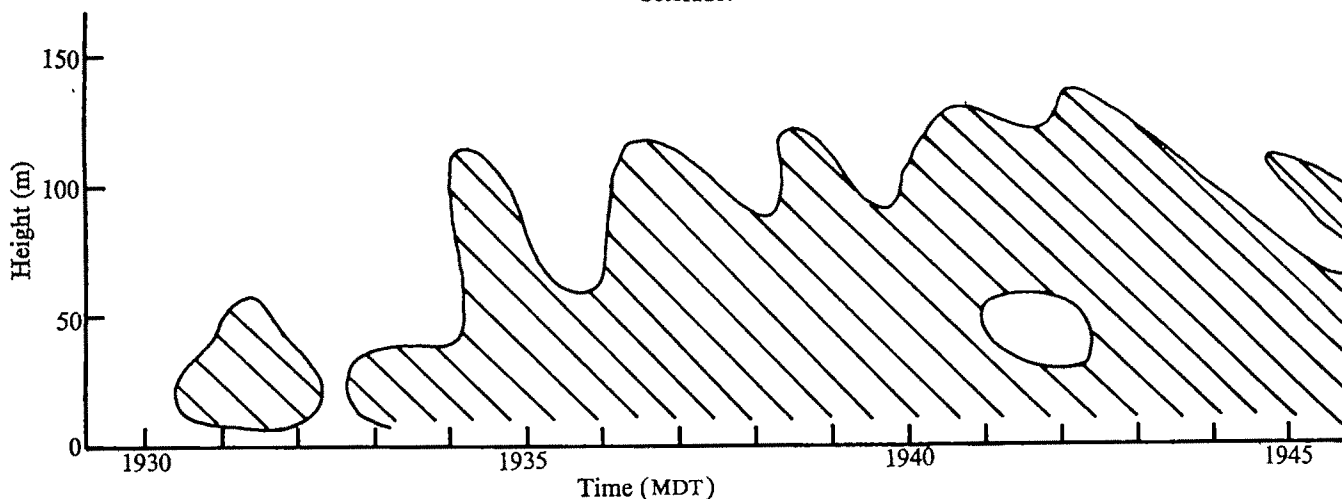
regions at 100 m are found to be $< 2^\circ$. The fluctuating nature of the shear at higher levels yields an undulatory pattern of shears $> 0.1 \text{ s}^{-1}$. Regions where this critical shear value is exceeded are shown in Fig. 3, indicating that even 16 km from this quite averagely sized prairie thunderstorm (with radar echoes extending to an altitude of 14.6 km), the clear air wind shear exceeded the safety limits consistently in the lower 100 m for > 10 min. Shortly after 1946 on August 5, a gust of more than 25 m s^{-1} destroyed two of the light weight propellers on the fast response sensors, or bivanes, eliminating further data.

The even more energetic density current of August 2, 1972 arose from a storm just 5 km away with radar tops observed to be above 17 km. Maximum velocities in this current exceeded 42 m s^{-1} , which quickly destroyed all bivanes on the tower. A recorded wind shear of 0.26 s^{-1} was observed over the 135-m separation of the two more rugged but slower response wind sensors. Thus the actual shear internal to the current (which otherwise might have been measured with 30-m height resolution provided by the bivanes) was probably greater than that recorded over the greater height increment separating the rugged sensors.

A model of density current wind shear

A feature common to the density current events observed was the drop in temperature with the arrival of the first gust. Empirically, it was found that the larger the temperature drop,

Fig. 3 Height against time plot of those regions where the wind shear exceeded 0.1 s^{-1} for the August 5, 1972 density current at Haswell, Colorado.



the greater the observed shear, consistent with the primitive equation model of gust fronts by Mitchell⁵. This suggested that temperature measurements relatively near the surface might serve as a quantitative predictor of the severity of density current shears. We now outline a simple model relating temperature drop to wind shear.

An obviously relevant parameter in density current modelling is the speed of the current, u_s . This is given by Turner⁶ as

$$u_s = \alpha [gH(T_a - T_g)/T_a]^{1/2} \quad (1)$$

where g is the acceleration of gravity, H is the depth of the current following the head, T_a the ambient air temperature, and T_g the temperature of the gust. Equation (1) comes from hydraulic theory. When dissipative processes are neglected the constant, α , is $\sqrt{2}$. Laboratory investigations yield an empirical value for α of 1.1 (ref. 3).

We consider first the shear along the upper boundary of the current near its nose. In this region of warm air overlying the colder current, we hypothesise that the turbulence generated there self-adjusts to gradient Richardson numbers, Ri (~ 0.2), as observed in thin inversion surfaces in the real atmosphere (Turner⁶, Hall *et al.*⁷). From the definition of Ri we write

$$\frac{\partial u}{\partial z} = \frac{1}{Ri} \frac{g}{\theta} \frac{\partial \theta}{\partial z} \quad (2)$$

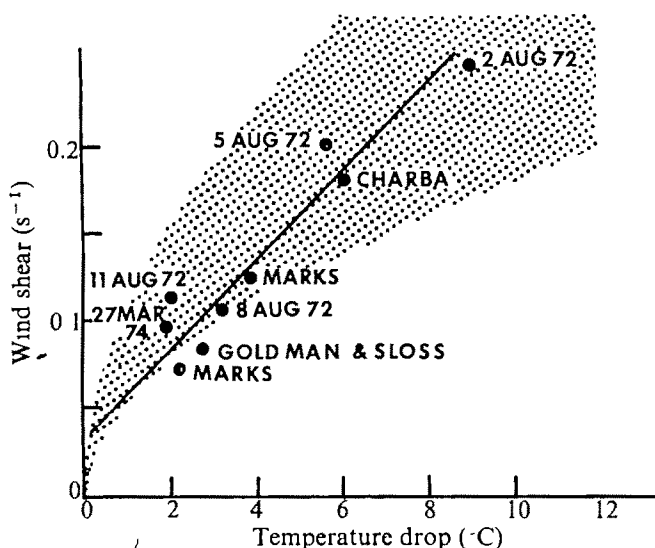
This can be approximated as

$$\frac{\partial u}{\partial z} \approx \frac{1}{Ri} \frac{g}{T} \frac{\Delta T}{\Delta u} \quad (3)$$

where the vertical gradients are assumed to occur over the same depths, the temperature lapse rates within and outside of the current are nearly equal, and $\Delta T = T_a - T_g$, measured near the surface. Assuming the ambient flow velocity is zero, $\Delta u = u_s$. Then equation (3) becomes

$$\frac{\partial u}{\partial z} = \frac{1}{\alpha Ri} \left(\frac{g \Delta T}{HT} \right)^{1/2} \quad (4)$$

Fig. 4 Measured wind shear plotted against temperature drop from the ambient atmosphere to the density current. The experimental data points, as described in the text, nearly fit a straight line, while the overlay stippled area represents approximate bounds on the shear predicted by equation (7).



Taking $H = 500$ m, which the sounder reveals to be a characteristic value for a number of such currents observed, $Ri = 0.2$, $\alpha = 1.1$ and $T = 300$ K, we have

$$\frac{\partial u}{\partial z} = 0.037(\Delta T)^{1/2} \quad (5)$$

For $\Delta T > 7^\circ\text{C}$ at the upper boundary of the current, equation (5) predicts wind shears > 0.1 , the safety limit derived by Snyder¹.

At the lower boundary of the current, where the shear is actually measurable on the tower, the speed must go to zero through some boundary layer thickness δ . We can then use equation (1) to estimate

$$\frac{\partial u}{\partial z} = \frac{u_s}{\delta} = \alpha \left(\frac{gH\Delta T}{\delta^2 T} \right)^{1/2} \quad (6)$$

Taking again $H \approx 500$ m and $50 \text{ m} < \delta < 100$ m (typically observed values) we have

$$0.044(\Delta T)^{1/2} < \frac{\partial u}{\partial z} < 0.089(\Delta T)^{1/2} \quad (7)$$

expressions surprisingly similar to equation (5), but predicting shears near the surface somewhat larger than at the upper boundary of the current.

A summary of the maximum observed wind shear for five density currents as recorded at Haswell is shown in Fig. 4, where the dated data points show the values recorded by bivanes on the 150-m tower, except for the August 2, 1972 event. Other points shown were recorded by Charba⁸ and by Marks⁹ at the WKY tower near Oklahoma City. The Goldman and Sloss¹⁰ data were obtained at the 150-m Kennedy Space Center, Florida tower. A least-squares linear fit to the data, relating wind shear to temperature drop observed in the density current, is shown as a solid line. The equation for this line is

$$\frac{\partial u}{\partial z} = 0.024 \Delta T + 0.03 \quad (8)$$

The area bounded by the limits of equation (7) is plotted as the stippled overlay which agrees well with the data. Equation (5) would plot near the lower bound of the stippled zone. The non-zero intercept in Fig. 4 probably reflects our neglect of the background shear.

The fact that the observed shear varies nearly linearly with ΔT may reflect an indirect dependence of H on ΔT as suggested in the two-dimensional primitive equation model of Mitchell⁵. Our simple heuristic model also avoids the variability in the static stability of the ambient air. Equation (7) is thus useful mainly to set approximate bounds on the shear using values of H typical of the few cases observed by the sounder.

If the simple relationships shown in Fig. 4 prove to be typical for an even larger data set, it would be a relatively simple matter to measure ΔT on a grid surrounding airports to warn of the wind shear produced by density currents moving across the area.

Conclusions

We conclude that even at distances > 16 km from thunderstorms, the wind shear in density currents from such storms can be a hazard to aircraft landing and taking off. Unless such density currents are delineated by blown dust, as in desert regions, they will be undetectable to the unaided observer. The temperature drop associated with these currents, however, gives a good measure of the wind shear to be expected. In addition, the ability of the acoustic sounder to detect such turbulent shear layers may provide new insight into the internal dynamics and structure of such currents in the atmosphere.

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Cell surface distribution of lectin receptors determined by resonance energy transfer

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The surface topography of concanavalin A (con A) bound to normal and transformed murine fibroblasts has been studied by a new technique involving fluorescence resonance energy transfer (RET). RET can provide a high resolution "map" of the distances separating con A-receptor complexes in single living cells. The distribution of con A is non-random in both normal and transformed cells, but sites are more closely approximated in the transformed. Approximation is induced by the con A but occurs at extremely slow rates indicating that the topography is not primarily determined by simple diffusion of complexes.

THE enhanced agglutinability of tumour compared with normal cells by plant lectins such as concanavalin A (con A) has led to the extensive use of lectins as "probes" of surface organisation. Yet the essential description of the binding of lectins to cell surfaces is controversial and incomplete. Although the number of binding sites per cell is equal^{1,2}, the surface density of bound con A is probably greater on transformed than normal cultured fibroblasts³. The distribution of con A over the surface may also be markedly different. The aggregation of con A-receptor complexes (C-R complexes) into clusters has been considered characteristic of the malignant cell⁴. The formation of these clusters depends on the movement of C-R complexes. When cells are prefixed with aldehydes and then labelled, the distribution of C-R complexes appears disperse or "random" on transformed as well as normal fibroblasts⁵. Thus C-R complexes are said to be more "mobile" on transformed cells. Similarly, after colchicine treatment, bound con A is distributed in large aggregates or caps on transformed but not on normal cultured fibroblasts⁶. These differences have been attributed alternatively to the intrinsic fluidity of the membrane or to submembranous structures, such as microtubules, which can modify the topography and apparent mobility of C-R complexes.

In our view, the available methodology has had two major limitations. First, the degree of topographical order has been difficult to quantify from electron micrographs. The terms "random", "dispersed" or "clustered" used to characterise the surface distribution of C-R complexes are not defined. Only marked degrees of clustering are obvious and subtle changes in topography cannot be determined. The assessment of order is further complicated by problems of statistical sampling of cells or regions of their surfaces.

Second, the distribution of C-R complexes on living cells has been studied only by fluorescence microscopy. This technique

permits direct observation of gross changes in C-R distribution ("random", large aggregates or caps) but does not allow observations at high resolution in dynamic conditions. Thus, the history of a given topographical distribution as seen on surface replicas by electron microscopy can only be reconstructed indirectly.

We describe here an approach to the analysis of surface topography that obviates some of these problems. We have utilised fluorescence resonance energy transfer (RET) between molecules of surface-bound con A to measure the distances separating them. The method makes possible the development of a high resolution "map" of the topography of C-R complexes. We have analysed the topography of C-R complexes on normal and transformed fibroblasts. The results provide a quantitative description of differences in the topographical organisation of C-R complexes between normal and transformed cells. The time course over which some of these differences evolve is very long and seems to exclude the rate of receptor diffusion as a major determinant of topographical organisation.

High resolution topographical mapping by RET

RET occurs when a donor chromophore which is excited by the absorption of light transmits some of the absorbed energy to another (acceptor) chromophore some distance away. This transfer does not involve the actual reabsorption of light by the acceptor but requires that the distance between the chromophores be relatively close (usually not exceeding 100 Å). Energy transfer varies most importantly as the inverse of the sixth power of the distance separating the chromophores. From the extent of transfer, quantitative theory allows an estimate of the distance between chromophores⁷. By labelling subpopulations of microtubule subunits or membranes with different chromophores, we have used the technique for the study of microtubule polymerisation and membrane-microtubule interaction *in vitro*⁸.

In the study reported here the con A used for cell labelling was a mixture of two populations separately conjugated to donor or acceptor fluorochromes. The ratio of acceptor to donor and the absolute dye to protein ratios were chosen such that at the surface density of bound con A an approximation of C-R complexes would be observed as an increase in RET.

Our system has two characteristics not frequently encountered in recent applications of RET. First, in our system the distance between donor and acceptor is not fixed. The chromophores are not placed at different sites on the same molecule as they are in applications in which RET can be used as a "spectroscopic ruler"⁹. Instead, because the chromophores are placed on different molecules statistically distributed over the two-

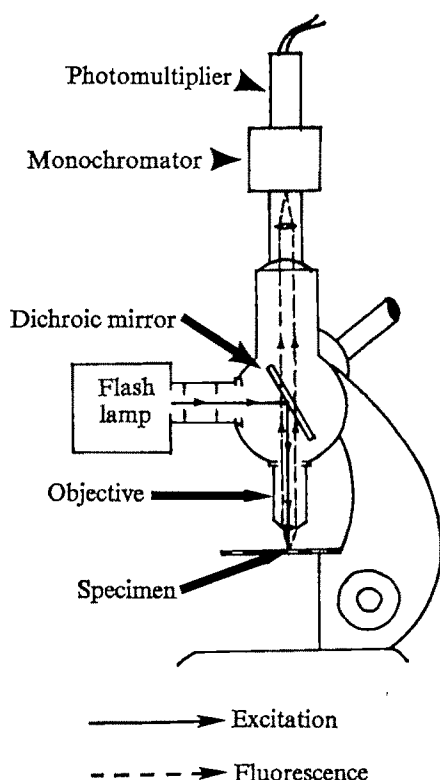


Fig. 1 Modified Zeiss microscope equipped for epiillumination. The incident light source was a Photophysics nanosecond flash lamp. The source was focused through a UG1 filter on to a Zeiss No. 450 dichroic mirror. Light was reflected downward and focussed on to the sample through the microscope objective (usually a planachromat 1.25 NA \times 100). Specimen fluorescence was collected in the objective, passed through the mirror, focussed on a grating monochromator and thence to a RCA 8850 photomultiplier. A monophoton counting device was used to record the light incident on the photomultiplier. A detailed description of the apparatus will be given separately (S.M.F., in preparation).

dimensional cell surface, the distance between chromophores varies in a corresponding statistical manner. An increase in RET thus corresponds to a statistical packing or aggregation of donors and acceptors. The analysis of this situation is not novel. RET between mixtures of chromophores in solution was the first system to be analysed by Förster⁷ and other physical chemists (for example, ref. 10). Second, in our analysis only changes in RET are considered useful. It has been emphasised repeatedly that the precise calculation of distances between donors and acceptors based on RET depends on the orientation and mobility of absorption and emission dipoles¹¹. This information is rarely available. We assume only that the orientation factors remain essentially constant throughout our experiment and focus attention on temporal variations of RET. Changes in RET represent changes in the distance separating donors and acceptors. We emphasise, however, that these relative distances correspond to changes of only a few Ångströms, for no energy transfer is detectable at separations exceeding 100 Å.

Observations were made with a modified fluorescence microscope using reflected light (epiillumination). This method of illumination provided sufficient intensity at the specimen when weak light sources (such as a flash lamp) were used. Fluorescence was detected using a sensitive monophoton counting device. The apparatus is shown in Fig. 1.

The ratio of acceptor to donor chromophores in mixtures used to label cells has proved critical. A model system in which this ratio is varied experimentally and related to the RET illustrates the principle and reveals several other features of our rationale. For these experiments, we used tubulin (tubulin and con A are approximately equal in molecular weight) which was

available in bulk and could be labelled conveniently to various ratios of dye to protein. We prepared mixtures of tubulin separately labelled with dimethylamino-naphthalene sulphonylchloride (DANS) or rhodamine isothiocyanate (RITC), and added various amounts of unlabelled tubulin. The proteins were cross linked with glutaraldehyde, concentrated and smeared on to glass slides for examination of fluorescence in our instrument. The precise calculation of RET from donor and acceptor fluorescence is a useful method for analysis of solutions and has been detailed elsewhere¹². Information on absorption by donor and acceptor and on acceptor quantum yield, however, is required and can be obtained only with great difficulty through the microscope. Since RET results in an increase in acceptor emission (sensitised fluorescence) and a decrease in donor fluorescence, we took the ratio of acceptor to donor fluorescence as an approximate measure of RET. This measure proved useful and, we feel, appropriate in view of our emphasis on relative changes and the uncertainties in obtaining less empirical expressions for RET. Figure 2 is a plot of the ratio of acceptor to donor fluorescence at different ratios of chromophores as a function of the mean distance between chromophores. As the distance increases, the ratio of fluorescence tends to a limiting value irrespective of the ratio of chromophores. This value is presumably characteristic of the shape of the donor fluorescence spectrum and any direct excitation of the acceptor and corresponds to the absence of RET. The existence of this limiting value enables one to set an empirical limit beyond which RET does not occur. Furthermore, the fluorescence ratio is more sensitive to changes in distance between fluorophores at high ratios of acceptor to donor. Thus for our experiments the ratio of acceptor to donor was set at about 5 to 1. Clearly at some still higher acceptor to donor ratio, RET from donor to acceptor will be essentially complete over a wide range of distances. Consequently RET gives poor resolution of donor to acceptor distances at both very low and very high ratios.

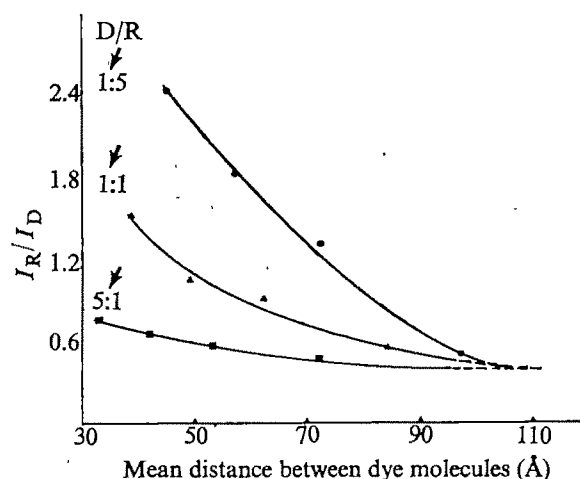


Fig. 2 Ratio of acceptor (rhodamine) to donor (DANS) fluorescence, I_R/I_D , in aggregated tubulin mixtures as a function of interfluorophore distance. The peak intensities at 570 nm and 515 nm were taken as a measure of acceptor and donor fluorescence, respectively. Tubulin was prepared by cyclic polymerisation by the method of Shelanski¹³ and labelled with DANS or RITC. Ratios of dye to protein were determined from the characteristic absorption at 350 nm and 553 nm, respectively, and protein by the method of Lowry¹⁴ using bovine serum albumin as standard. The separately labelled tubulin was mixed to give the desired acceptor to donor ratio and diluted with unlabelled tubulin in various amounts. The mixtures were then incubated with 20% glutaraldehyde in the cold overnight, precipitated with acetone and washed in media. The cross-linked precipitate was then smeared on a glass slide under a coverslip and examined in our instrument. The mean distance is calculated between all species of dyes taking each fluorophore as a point source and the density of the hydrated precipitate as 1.0.

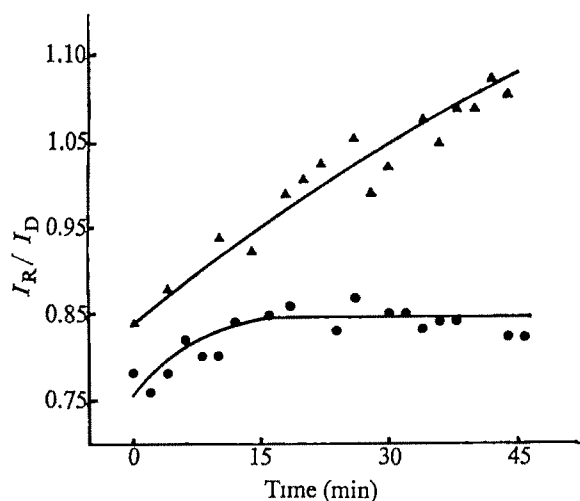


Fig. 3 Ratio of acceptor (rhodamine) to donor (DANS) fluorescence, I_R/I_D , from con A-labelled murine fibroblasts as a function of time of incubation at room temperature. The ratio of rhodamine to DANS was 5 to 1, the ratios of dye to con A protein were 3.8 to 1 for DANS-con A and 7 to 1 for RITC-con A. Total con A labelling concentration was $180 \mu\text{g ml}^{-1}$. Cells were labelled at 4°C for 1 h as described and excess dye was removed. Observations on the microscope stage at room temperature were commenced immediately after removal of dye. Cells were located by phase contrast using green filtered light. The flash lamp used to excite the specimen did not bleach the dye. In fixed specimens identical readings could be obtained for hours. Thus, membrane or other damage which could result from the high light intensities associated with bleaching (that are obviously essential for the photobleaching technique)²⁴⁻²⁶ is minimised. Δ , SV3T3 cells; \bullet , 3T3 cells.

Surface topography of con A-labelled fibroblasts

The application of the technique to living 3T3 and SV40-transformed 3T3 fibroblasts is partly confirmatory of previous studies of con A surface topography: C-R complexes are more clustered on transformed than normal cells^{4,5}. In addition, several features of the dynamics of C-R interaction are revealed, and the distribution of C-R complexes on 3T3 is shown to be non-random.

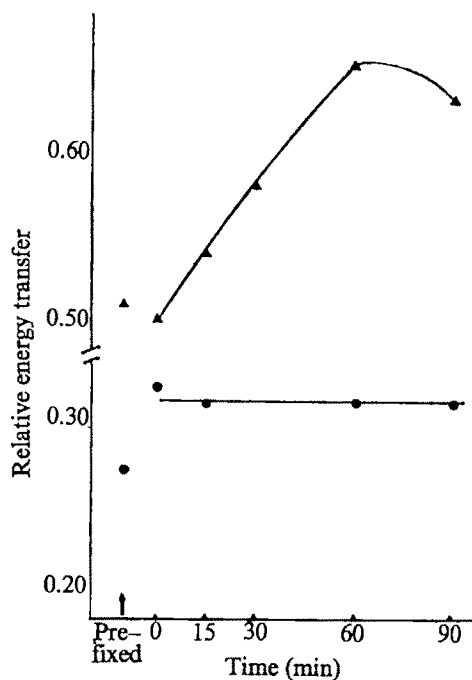
Con A was labelled with DANS (as the cycloheptaamylose complex)¹⁶ or RITC and purified by affinity chromatography as previously described¹⁶. Fibroblasts were grown in modified Dulbecco's medium with 10% calf serum on glass coverslips. Monolayers were labelled with mixtures of DANS-con A and RITC-con A at 4°C for 1 h. After rinsing away excess dye, the coverslips (fixed or unfixed) were inverted, sealed with paraffin on to glass slides and transferred to the instrument microscope stage. Cell-associated fluorescence was observed at intervals during incubation at room temperature. Data from single representative 3T3 and SV3T3 fibroblasts are shown in Fig. 3. Both cell types show an increase in RET with incubation. The relative change on 3T3, however, is quantitatively smaller than on SV3T3, and is completed after 15 min of incubation. We conclude that the topographical distribution of C-R complexes on SV3T3 is not random but also that the distribution on 3T3 is not completely random or disperse (although quantitatively the complexes are less clustered than for SV3T3). C-R complexes undergo a limited and rapid approximation on 3T3. By including large segments or even the entire cell in the field the average behaviour of C-R complexes over the whole region is recorded. Because with prolonged culture 3T3 spontaneously lose contact inhibition, we also studied C-R complexes on primary mouse fibroblasts and obtained results identical to those with our strain of 3T3.

Figure 4 shows an experiment in which the monolayers were fixed with paraformaldehyde after incubation at 37°C for

various times. The fixation allowed longer periods for accumulation of fluorescence photons and so there is less scatter of the data points. The same basic features are observed, indicating that fixation did not alter the topography of C-R complexes. At 37°C the aggregation of C-R complexes on 3T3 fibroblasts is completed by the time the first observation can be made at "0" min. But when cells are prefixed and then labelled, the RET obtained shows clearly that the time required for labelling at 4°C and subsequent fixation has allowed significant approximation of C-R complexes. Figure 4 again shows that the relative change of RET on SV3T3 is large but that the change occurs over 1 h even at 37°C .

An alternative method for estimation of RET is based on measurement of the lifetime of the excited state. RET depletes the population of excited donors and thus shortens the lifetime. If a nanosecond flash lamp is used as the exciting source, the lifetime of the excited state can be determined for the label on single cells. The techniques for data collection and analysis have been detailed elsewhere¹⁷. The results obtained from fixed preparations using this method agree qualitatively with the spectral data. Unfortunately, each determination of lifetime requires many minutes of photon counting so that the method was not suitable for observations of living cells nor for surveying large numbers of cells. Additional information on the distribution of bound con A, however, can be obtained from analysis of the fluorescence spectra taken at intervals during the lifetime of the excited donor population (so-called time-resolved spectra). For donors and acceptors separated by statistically variable distances the probability of RET between a donor and the population of acceptors that surround it will also vary. (This is in contrast to the situation in which donor and acceptor are separated by a fixed distance on the same molecule.) Consequently, immediately after excitation the probability of RET will be very high between close donors and acceptors and will decline rapidly as the remaining donors are separated from acceptors by greater distances. In Fig. 5 time resolved spectra from SV3T3 are recorded. The peak at 515 nm corresponds to DANS (donor) emission and decays with time as the excited state is depleted. The second peak at 570 nm results from the

Fig. 4 Ratio of acceptor to donor fluorescence, I_R/I_D , from con A-labelled cells against time. Conditions were as for Fig. 3, except that cells were fixed with 2% paraformaldehyde after incubation but before measurements of fluorescence. Incubation was at 37°C . "Prefixed" specimen was fixed for 20 min at 37°C before labelling. Δ , SV3T3 cells; \bullet , 3T3 cells.



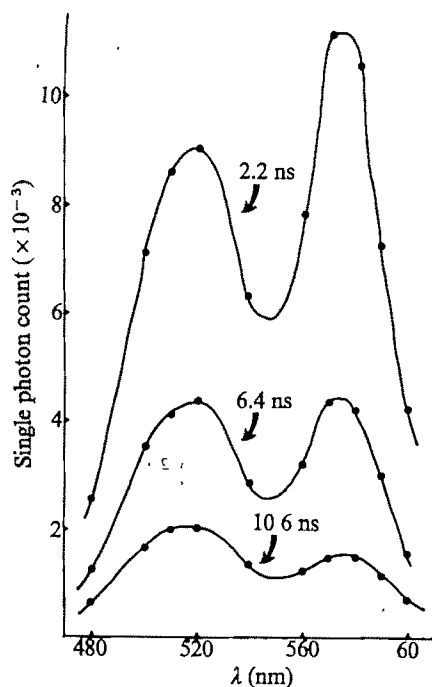


Fig. 5 Time resolved spectra from con A (DANS, rhodamine)-labelled SV3T3 fibroblasts. Photon counts were accumulated at various indicated times after the peak of the light pulse used for excitation. At 2.2 ns RET is high between DANS and nearby RITC. The DANS fluorophores that remain excited at later times are those which are statistically distributed at greater distances from the RITC and thus are less efficient in RET.

rhodamine fluorescence. The relative peak heights of fluorescence, rhodamine/DANS (acceptor/donor) are greatest early during the lifetime of the excited state in accord with the statistical surface distribution of the labelled con A.

The contribution of internalised (pinocytosed) con A to our measurements is probably not significant. We assessed the internalisation by determining the amount of con A that could not be eluted by the competing hapten sugar, α -methylmannoside. For these purposes radioactive (125 I-labelled) con A was used, for at intervals of less than 60 min the amount of internalised fluorescent label gave unreliably low signals. The amount of radioactivity that could not be eluted from dense monolayers in the conditions of our experiments was <10% of the total.

Inherent distribution of con A receptors

In agreement with observations made using other techniques, the approximation of receptors on SV3T3 is significantly greater than on non-transformed 3T3 or primary mouse fibroblasts^{4,5}. In addition, we find that the RET on prefixed SV3T3 is consistently higher than for prefixed 3T3 (example in Figs 3 and 4). This could be because at the high level of resolution afforded by the technique con A receptors are inherently more clustered on SV3T3, that is some of the approximation of C-R complexes on SV3T3 is not induced by the con A. Such an interpretation runs contrary to a large body of evidence using other techniques, indicating the inherent distribution to be disperse on all cell types but it should be emphasised that the degree of resolution afforded by RET exceeds that available by other techniques. Alternatively the surface density of con A receptors on SV3T3 may be higher than for 3T3. Note that this interpretation does not depend on measurements of the actual surface area. This conclusion was originally suggested by Inbar and Sachs¹⁸ and Noonan and Burger¹⁹ but has been difficult to test because of complications in the measurement of cell surface area. Using scanning electron microscopy, Collard and Temmink³ have attempted to account for variations in surface contour and

calculated a significantly greater density of con A receptors on SV3T3.

At the high resolution afforded by RET the topographical distribution of C-R complexes is not random on non-transformed 3T3 or primary mouse fibroblasts. Using the haemocyanin-con A and ferritin labelling techniques, we and others^{4,5} have reported the distribution of C-R complexes to be disperse or random. This conclusion was inferred largely from differences between normal and the more obvious clustered C-R aggregates on transformed mouse fibroblasts. Other groups have not found consistent differences between normal and transformed murine cells²⁰⁻²². Differences have been attributed to variations in cell line and to methodology. Our findings with RET do not completely resolve the issue since the high resolution obtained with RET cannot be compared directly with the topographical descriptions obtained by other techniques. In addition RET provides semiquantitative information integrated over large areas of the surface. Our results, however, suggest that topographical differences between normal and transformed murine fibroblasts are essentially quantitative.

Movement of C-R complexes

In agreement with previous observations involving C-R movement over long distances^{5,23} the non-randomness observed by RET on all cell types is induced to some degree by the con A itself, for prefixed cells or early time points show lesser degrees of RET.

The aggregation of C-R complexes on 3T3 cells is completed in a relatively short time compared with SV3T3 cells. Whether this is because the end point of aggregation represents a lesser degree of organisation or because the organisation is attained more efficiently in the 3T3 cannot be determined from the data reported here.

Quantitative aspects of this slow movement imply that the organisation of C-R complexes observed by RET is not limited by their lateral diffusion over the surface. We re-emphasise that the changes of RET observed require movements of only Ångströms, perhaps 100 Å at the most. A rough calculation of the diffusion coefficient (D) for C-R complexes can be obtained from our data using a well known expression relating the relaxation time of diffusion (τ) to the mean distance (ΔX) travelled by the complexes ($\Delta X^2 = 2\tau D$). For these purposes we can take τ as the time required to attain plateau levels of RET and ΔX to be between 10 and 100 Å. The range of D can then be calculated to be between 10^{-16} and 10^{-17} cm² s⁻¹. This is exceedingly small compared with all previous measurements of movements of antigen-antibody complexes or of succinyl-con A-receptor complexes. For example, experiments²⁴⁻²⁶ utilising the recovery of fluorescence after photobleaching have yielded diffusion coefficients of the order of 10^{-10} cm² s⁻¹ for succinyl con A on fibroblasts (even this is considerably lower than rhodopsin in photoreceptor membranes, 4×10^{-9} cm² s⁻¹). It is interesting that in the experiments of Jacobson *et al.*²⁵ and Schlessinger *et al.*²⁶ recovery from photobleaching was not complete in minutes and suggested a subpopulation of "immobilised" receptors. Even if we assume that the true diffusing species is a con A-cross-linked aggregate involving all bound con A (approximately 10^7 molecules per cell) the diffusion rate, which varies as the square root of the molecular weight, could not be lowered by 6 to 7 orders of magnitude. We suppose instead that the change in RET is not diffusion limited, but represents a secondary organisational change that involves some other mechanism, perhaps the interaction of submembranous structures (microtubules, microfilaments or spectrin-like molecules) with the cross-linked C-R complexes.

Future applications

The extension of RET to the study of the distribution of other surface constituents is obvious. We have reduced the analysis to an empirical semiquantitative one which provides considerable information without forcing assumptions or extensive instrumental corrections that are unreliable and cumbersome. Thus

the method provides a relatively straightforward and reproducible means for obtaining information on distances separating any exogenous ligand that can be labelled with donor and acceptor fluorophores without loss of activity. Distribution can in general be studied in living or fixed material. We have used average signals from large areas of the cell surface. Similar data, however, can be obtained from areas of the surface of as little as 2 μm in diameter, and so regional surface differences can also be compared.

The results obtained with con A serve to direct attention away from the concept of simple unrestricted mobility of surface complexes in the membrane lipid matrix. We predict that RET studies with other ligand-receptor complexes will support the view that cell-surface topography is determined to a large degree by subtle interactions between linked receptors and changes in submembranous elements induced by them.

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Crystallographic structure studies of an IgG molecule and an Fc fragment

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The crystal structures of a human IgG antibody molecule Kol and a human Fc fragment have been determined at 4-Å and 3.5-Å resolution respectively, by isomorphous replacement. The electron-density maps were interpreted in terms of immunoglobulin domains based on the Rei and McPC 603 models (Kol) and by model-building (Fc). The Fab parts of Kol have a different quaternary structure from that observed in isolated crystalline Fab fragments, there being no longitudinal V-C contact in Kol. The Fc part C terminal to the hinge is disordered in the Kol crystals. It is suggested that the Kol molecule is flexible in solution, whereas fragments are rigid. In the Fc fragment both C_{H3} and C_{H2} show the immunoglobulin fold. The C_{H3} dimer aggregates as C_{H1}-C_L while C_{H2} are widely separated from each other. The carbohydrate bound to Fc is in fixed position. From these structures a hypothetical liganded antibody molecule has been constructed, which is assumed to be rigid.

ANTIBODY molecules serve a dual function: to recognise foreign cells and macromolecules and to trigger the events leading to their elimination. Specific recognition requires surface structures complementary to the antigen and hence a huge variety of antibody molecules. In contrast the effector functions would most economically be performed if all antibody molecules were identical. An antibody molecule of the IgG class consists of two identical light (L) and two identical heavy (H) chains^{1,2}. Each of these polypeptide chains can be subdivided into a constant

region with an amino acid sequence identical to other chains of the same class and a variable region whose sequence varies between antibodies specific for different antigens. Antibody functions are located on different parts of the molecule with recognition lying in a fragment consisting of a light chain and half of the heavy chain—the Fab fragment. Effector function on the other hand resides in the C-terminal half of the heavy chain—the Fc part. There is, however, cooperativity, and effector functions are triggered by antigen binding. How this signal is transferred is open to debate and in this article we will discuss a hypothetical model based on our current knowledge of three-dimensional antibody structure.

Several crystal structures of immunoglobulin fragments (Fab fragments and Bence-Jones proteins) have been analysed during the last few years (for a review, see ref. 3). From the recent crystal structure analyses of a complete human IgG antibody Kol⁴ and a human Fc fragment^{5,6} a coherent view of antibody structure is beginning to emerge.

The crystallographic analysis of the Kol protein was pursued to 5-Å resolution by isomorphous replacement⁴ and later extended to 4-Å resolution. The electron-density map was analysed in terms of domain structure making use of the known structure of the V (variable) and C (constant) parts of the Fab fragment McPC 603³ and the Rei fragment⁷. It was evident that there are differences in domain tertiary structure between the Kol protein and McPC 603. We have not yet tried to analyse these in detail, but it is clear that they do not affect the later discussion on longitudinal domain contacts. A tentative interpretation in the 4-Å map of the C^α chain folding is given for the heavy chain from residue 213 to the -Cys-Pro-Pro-Cys- segment, and for the light chain from residue 209 to the heavy-light chain disulphide (amino acid sequences in the Kol Fab part refer to the McPC 603 molecule³ and to the Rei protein V_L dimer⁸⁻¹⁰). The Fc fragment crystal structure was

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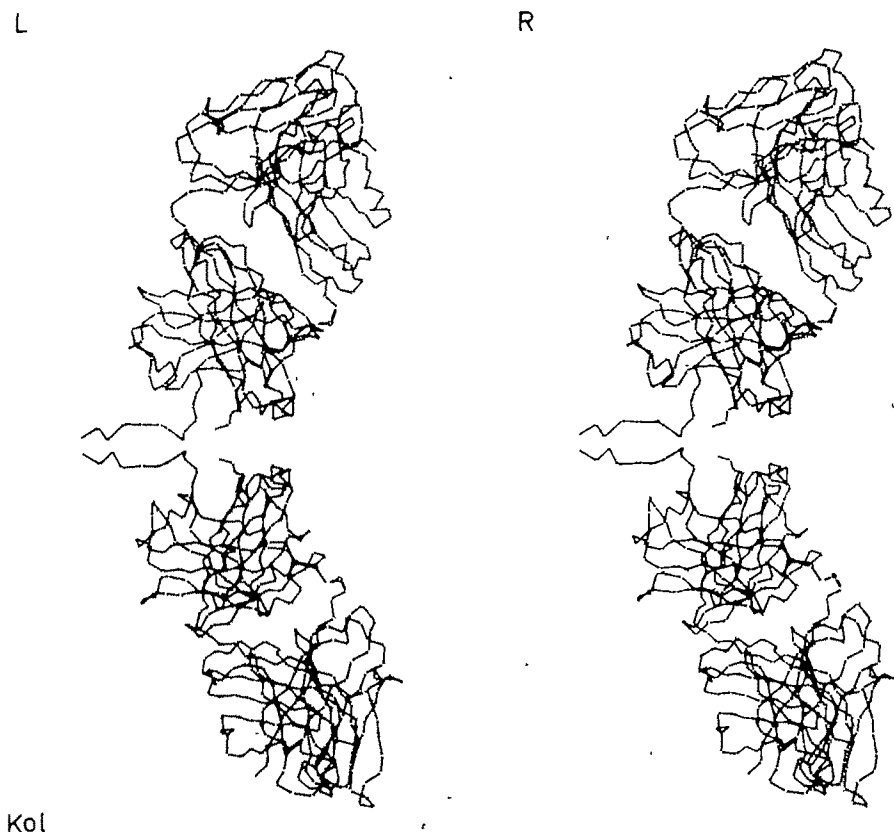


Fig. 1 Stereo drawing of the Kol (Fab)₂ part C*; atom positions were derived by fitting the McPC 603 (ref. 3) V and C dimers to the Kol electron-density map. The coordinates of the C-terminal residues of heavy and light chains and the hinge peptide were obtained by interpretation of the 4-Å Fourier map.

Kol

analysed at 4-Å resolution⁵ and later at 3.5-Å resolution⁶, using isomorphous phases and a complete model was constructed.

The Kol molecule

The C* carbon atom positions of the (Fab)₂ part of the Kol molecule are shown in the stereogram, Fig. 1. The Fab arms subtend an angle of 125°, and the tips of the Fab arms are about 146 Å apart. Except for slight differences in the domain tertiary structure, the V_L-V_H and C_L-C_{H1} dimers appear to be indistinguishable between Kol and McPC 603. In particular, the geometry of aggregation within V and C dimers is very similar. There is a difference, however, in the relative orientation of V and C dimers which can be described by a rotation about an axis through the switch peptides at residues 118-119 (heavy chain) and 109-110 (light chain) (Fig. 2). (Switch peptides are the segments connecting V and C parts comprising residues at 110 (light chain) and 119 (heavy chain).) This, of course, requires a conformational change in the switch peptide segment, which might be accomplished with minimal perturbation as the segment is in an extended conformation. Figure 2 compares the McPC 603 Fab fragment and the Kol Fab part as seen down the switch peptide rotation axis. V_L and V_H, as well as C_L and C_{H1} are related by local diads. These diads subtend an angle (Fab angle) of 135° in the McPC 603 fragment and 170° in the Kol protein, demonstrating the major change in quaternary structure: 'bending the elbow'.

It is already evident from Fig. 2 that in the McPC 603 molecule some residues of V_H and C_{H1} come close to each other. There are 6 C*-C* distances smaller than 6.5 Å (formed by segments 8-10 in V_H and 152 and 207-208 in C_{H1}) and 41 below 10 Å. There are, of course, several contacts between residues of the switch peptide and C_{H1} not included. We believe that these are irrelevant for the later discussion. Equivalent residues of V_L C_L of the light chain adopting the heavy chain conformation are in contact in the Bence-Jones protein dimer structure (ref. 11 and A. Edmundson, personal communication). In contrast, due to the asymmetry of the molecule, V_L and C_L are much further separated in the McPC 603 molecule. There are 2 C* distances below 6.5 Å and 28 below 10 Å.

Through the widening of the Fab angle from 120° to 170° in Kol there is no contact between C* atoms of V_H and C_{H1} smaller than 7 Å. Five distances are below 10 Å. Also, V_L and C_L have no contact (six distances below 10 Å). For comparison, the lateral V_L-V_H and C_L-C_{H1} contacts in the McPC 603 Fab fragment involve nine and seven C* distances respectively closer than 6.5 Å and about 120 distances below 10 Å for both contacts. The C_{H3}-C_{H3} contact in Fc involves 14 C* distances smaller than 6.5 Å and about 150 below 10 Å. According to this admittedly very crude criterion, there is a strong V_H-C_{H1} interaction in crystalline Fab fragment structures while there is no appreciable longitudinal interaction in the Kol Fab parts. In rabbit light chains a disulphide bond links V_L and C_L residues 80 and 172 (ref. 12). A sufficiently close approach in the Kol model occurs between C* atoms of residues 81 and 171 of 9 Å. This distance is 7 Å in the McPC 603 model. The disulphide linkage therefore does not oppose the Fab angle ('elbow') bending considerably. The two Fab arms in the Kol protein have no contact with each other except the covalent linkage of the hinge. There is no approach of C* of the two C_L domains closer than 12.5 Å.

No interpretable electron density is found in the Kol Fourier map which could be assigned to the Fc part⁴. The Fourier map contains significant density in that region where the Fc stem must be located with peak heights about 2.5 times the r.m.s. error in electron density. This is to be compared with the density in the Fab parts with peak heights about four times the error level. We have concluded that the Fc stem is disordered in the Kol crystals, C-terminal to the hinge peptide. Indeed, the (Cys-Pro-Pro-Cys)₂ peptide of the hinge on the molecular and crystallographic diad is only 50 Å away from the crystallographic 3₂ axis. The Fc fragment molecule is too long and too thick to be accommodated on the diad axis^{4,6}. It would interfere with crystallographically related Fc parts. On the other hand, the very close packing in the Kol crystal structure around the hinge peptide (see Fig. 6 in ref. 4) does not allow a close approach of the C_{H2} domains of Fc towards (Fab)₂, but requires a rather extended hinge segment (the hinge peptide is the segment connecting C_{H1} and C_{H2} and containing the inter-heavy chain disulphide linkage).

We constructed a 'minimum disorder' model of the Kol molecule. The Fc fragment structure, when combined with the Fab parts in the Kol crystal structure, should deviate minimally from the Kol crystal symmetry and not violate the crystal packing. The Fc fragment model was placed with its local diad on the crystallographic diad of the Kol lattice so that the hinge peptide, as seen in the Kol molecule and as surmised in the Fc fragment (see later), overlapped. Fc was then rotated azimuthally so that there was minimal overlap with (Fab)₂ parts from crystallographically related molecules. It had to be shifted by about 15 Å along the diad, away from the Fab parts, to avoid overlap. Here it would penetrate the 3₂ axis and interfere with crystallographically related Fc parts. Fc was therefore bent about an axis close to the top of the molecule and perpendicular to the a b plane of the Kol lattice minimally, to avoid this steric interference. It is obvious that these three operations are not independent. Apparently, a correct but intractable three-dimensional treatment would not change the main aspect of the model in a substantial way. Figure 3 represents the model. Evidently there are no contacts between (Fab)₂ and Fc, except the covalent linkage through the hinge peptide, which must have a rather extended conformation. In the 'minimum disorder' model the wide separation of (Fab)₂ and Fc requires this piece of chain to be stretched out. These observations of absent longitudinal interactions between V_H and C_H1 and between C_H1 and C_H2, as well as missing lateral interactions between both C_L parts in the Kol molecule in comparison to the existing V_H-C_H1 interactions in free Fab fragments, will be important in later discussion.

Fc fragment structure

Figure 4 is a stereo plot of the C^α carbon atom positions of the Fc fragment. The molecule has the shape of a Mickey Mouse with the compact C_H3 dimer forming the head, and the C_H2 domains forming the ears. The C_H3 dimer pairing is closely similar to the C_H-C_L pairs found in Fab fragments^{5,6}. C_H2 and C_H3 show the common immunoglobulin fold. They are connected by a loosely folded segment from Ser (337) to Gln (342) which is susceptible to proteolytic attack^{13,14} (amino acid sequence numbers in the Fc fragment are based on the Eu amino acid sequence¹). The longitudinal C_H2-C_H3 contact is characterised by 3 C^α distances less than 6.5 Å (and 43 below 10 Å), not considering contacts made by the connecting peptide. It comprises the segments 247-253 and 310-314 (C_H2) and 376-379 and 428-433 (C_H3) respectively. These segments are homologous to those mediating the V_H-C_H1 contact in Fab fragments described before. The electron density at the C terminus fades away after Ser (442), indicating mobility from this residue on. The N terminus seems to be folded back from Pro (238) on and in intimate contact with the carbohydrate moiety discussed below. This mostly unresolved contact makes an interpretation of the peptide chain conformation around the hinge region difficult at present. A possible chain tracing has

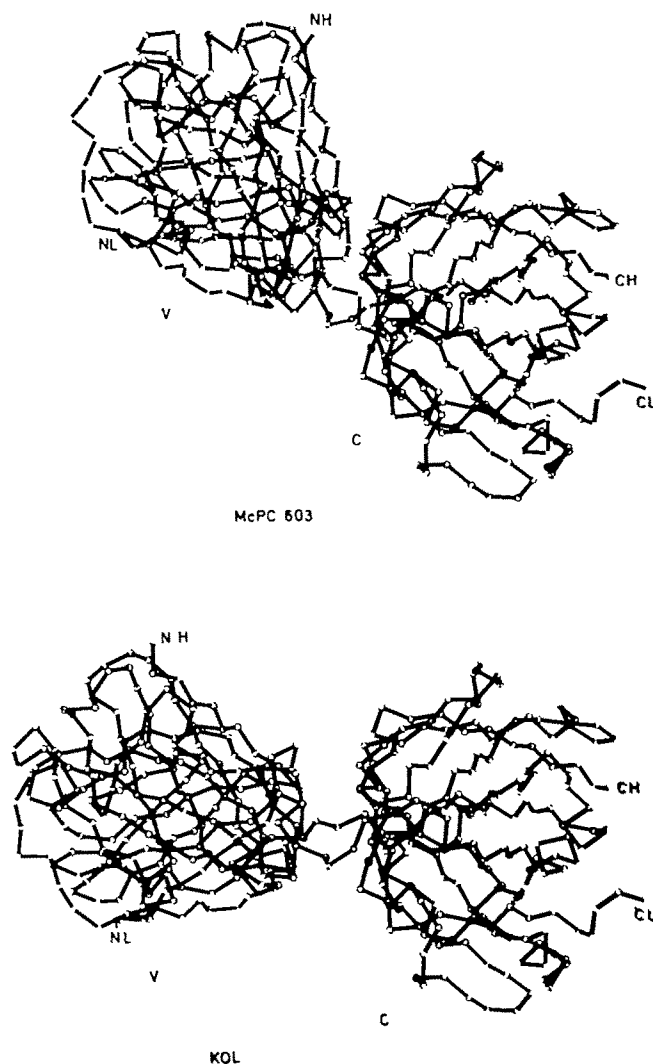


Fig. 2 Comparison of McPC 603 Fab fragment and Kol Fab part. The Fab angle changes by 40°. There is a V_H-C_H1 contact in the fragment, but not in the intact molecule. NH, NL: N terminus of heavy and light chain, respectively. CH, CL: C terminus of heavy and light chain, respectively.

the segment from Gly (236) to Pro (232) in contact with one branch of the carbohydrate chain. The (Cys-Pro-Pro-Cys)₂ segment seems to be disordered. This is not unexpected, as in the Kol crystals the (Cys-Pro-Pro-Cys)₂ segment is fixed while the Fc part is disordered. The electron density assigned

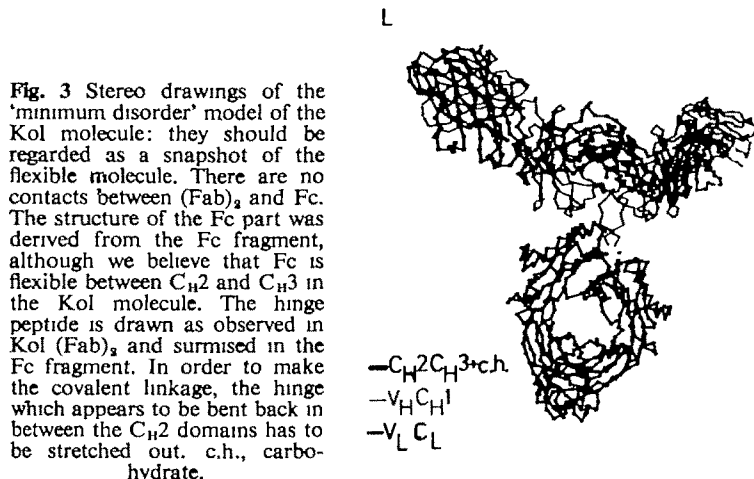


Fig. 3 Stereo drawings of the 'minimum disorder' model of the Kol molecule: they should be regarded as a snapshot of the flexible molecule. There are no contacts between (Fab)₂ and Fc. The structure of the Fc part was derived from the Fc fragment, although we believe that Fc is flexible between C_H2 and C_H3 in the Kol molecule. The hinge peptide is drawn as observed in Kol (Fab)₂ and surmised in the Fc fragment. In order to make the covalent linkage, the hinge which appears to be bent back in between the C_H2 domains has to be stretched out. c.h., carbohydrate.

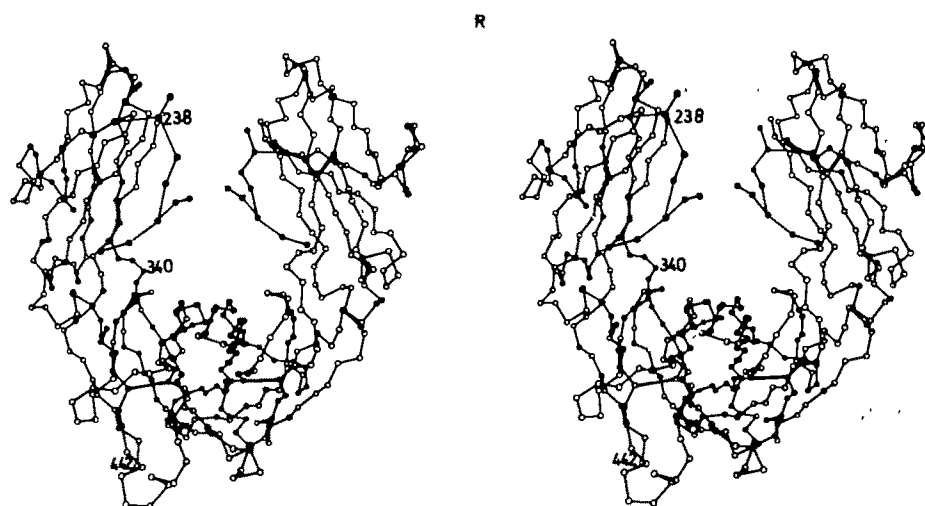
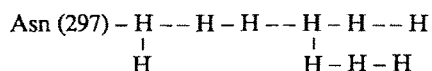


Fig. 4 Stereo drawing of the C^α carbon positions and the centres of the carbohydrate hexose units of the Fc fragment. The carbohydrate structure was assumed as described in the text. ●, Approximate centres of carbohydrate hexose units. The carbohydrate attachment site is Asn (297). ○, C^α carbon positions. The disulphide linkages are indicated.

to the bound carbohydrate could accommodate a branched carbohydrate chain of the following arrangement.



(H is a hexose unit; at three positions (--) we had difficulties in keeping linked residues in density. The present model is not connected at these positions.)

Such a chain would differ from some carbohydrate sequences of myeloma proteins¹⁸. We have to expect inhomogeneity of the carbohydrate in our material which might make the detailed analysis difficult. The crystallographic analysis of the carbohydrate structure requires further structure refinement. The carbohydrate moiety covers a large part of the C face of the C_{H2} domain.

Our model indicates that the carbohydrate covers apolar residues on the C face, among these being: Phe (241), Phe (243), Val (262), Val (264), Val (266). The site of attachment of the carbohydrate moiety at Asn (297) is part of a reverse turn. The four residues in the turn are Tyr-Asn-Ser-Thr. In general, carbohydrate attachment preferentially occurs at amino acid sequences X-Asn-X-Thr(Ser) (see review, ref. 16). Pancreatic trypsin inhibitor (PTI) and ribonuclease are molecules of known three-dimensional structures¹⁷⁻¹⁹. There exist closely related variants of these molecules (colostrum inhibitor²⁰ and ribonuclease B²¹) which carry carbohydrate. The carbohydrate site is part of a bend in all cases. The detailed conformations are very different, however.

Domain tertiary structure comparison

A set of comparative drawings of the individual domains in the Fab fragments McPC 603 and New, and the Bence-Jones proteins Mcg and Rei has been published³. A detailed comparison with the chain folding in the (Fab)₂ part of the Kol molecule has to await higher resolution data of the Kol crystals.

Comparative drawings of the chain folding of V, C_{H1} , C_{H2} and C_{H3} domains have been prepared and are shown in ref. 6. The most prominent differences between V and C domains lie in loops mediating V-V and C-C aggregation respectively. According to these criteria, it is clear that Fc C_{H3} has the characteristics of a C domain: the long loop involved in the C-C interface is present with the segment 398-403 forming the corner. V-characteristic loops are absent. C_{H2} , however, has some of the features of a V domain: the V characteristic loop is present, but shorter than in V domains. The C characteristic loop is also present, but shorter than in C domains and longer than in V domains. This raises the interesting question of the evolutionary origin of the C_{H2} segment. While amino acid

sequence homologies have been interpreted as indicating a close relation of C_{H2} to other C segments and only a distant relation to V segments, the structural comparison points to an intermediate structure. There is a remote possibility that the type of aggregation influences chain folding, the C_{H2} conformation being characteristic of a free domain, and the other C segments being deformed by their C-C contacts.

Domain-domain interactions

(1) Lateral interactions. The lateral V-V (V_H - V_L , V_L - V_L) and C-C (C_L - C_{H1} , C_L - C_L) interactions observed in Fab fragments^{22,23} and Bence-Jones proteins^{10,24} have been described in great detail and, will not be considered here. The relative arrangement of the two C_{H3} domains observed in the Fc fragment is closely similar to the C_L - C_{H1} aggregation observed in McPC 603. The C_{H2} domains have no contact with each other except the covalent linkage through the hinge. It is an intriguing question why C_{H2} forms neither the familiar C-C nor V-V contact. It has been suggested that the tertiary structure of the C_{H2} domain does not resemble a C but also lacks some features of a V domain. This might prevent dimerisation. In the presence of the spatially fixed carbohydrate moiety formation of the C-C contact is impossible. Furthermore, some of the hydrophobic residues in C_{H3} , which lie close to the local diad, and are probably important for the contact, are replaced by polar groups in C_{H2} . An analogous argument holds against a potential V-V contact.

(2) Longitudinal interactions. In the Kol protein and the 'minimum disorder' model derived from it, no longitudinal contacts are observed between V_H and C_{H1} , V_L and C_L , and C_{H1} and C_{H2} . There are also no contacts between the Fab arms. In view of this lack of stabilisation along the chain, it is difficult to assume that the Kol molecule should be rigid in solution. We rather presume that flexibility exists between V and C dimers ('elbow' V-C flexibility), between the Fab arms ((Fab)₂ flexibility) and between Fc and (Fab)₂ (Fc-(Fab)₂ flexibility). As mentioned before, flexibility between Fc and (Fab)₂ could explain the weak, uninterpretable electron density for Fc in the Kol crystals. The relative positions of V and C dimers and Fab arms are frozen in the Kol crystal lattice dictated by crystal packing requirements. Even the C_{H2} segments might sit flexibly on the C_{H3} dimers (C_{H2} - C_{H3} flexibility) in the Fc part in the Kol protein in contrast to their fixed position in the fragment. We have, of course, no evidence for this as the Fc part in the Kol crystals is not yet analysed. Rigidity without longitudinal contact between the domains could only be brought about if the switch peptides and the hinge peptide adopted rigid conformations. These segments are in an extended conformation, largely exposed to solvent. Inherent rigidity of these peptide chains is improbable.

The model which we would like to propose for the Kol protein and for other unliganded IgG molecules is a highly flexible one.

The degree of segmental flexibility appears more limited for V-C ('rotation' about 'switch axis') than for (Fab)₂ or Fc-(Fab)₂, which are connected by far longer extended polypeptide chains.

In obvious contrast, the Fab fragments and the closely similar Bence-Jones protein dimer appear to be rigid molecules. In the three structures, V and C dimers subtend a similar Fab ('elbow') angle of 120° to 135°. Crystal packing is different in the three crystal structures and would be unlikely to have the same effect on the Fab angle if it were variable. Indeed, a longitudinal contact exists between V_H and C_{H1} in these fragments as described before. This contact surface is smaller in size than the lateral C-C or V-V contacts, but it has been found in other systems that small contact surfaces may mediate strong binding (PTI-inhibitor-trypsin²⁵). In the Fc fragment C_{H2} and C_{H3} form a contact as described. The linking peptide is not in a completely extended conformation but is irregularly bent between residues Lys (338) and Gly (341). The presence of two nearly identical contacts in crystallographically non-identical environment speaks for a rigid Fc fragment, although it would be desirable to confirm this by structure analyses of different crystal forms.

It is remarkable and deserves further consideration that homologous segments mediate the close V-C contacts in the Fab fragment and the close C_{H2}-C_{H3} contacts in the Fc fragment: V_H, residues 8-10; C_{H1}, residues 152 and 207-208 (McPC 603); C_{H2}, residues 247-253 and 310-314; C_{H3}, residues 376-379 and 428-433.

These contacts have been inferred from C^{*} distance calculations. The actual bonding interactions are, of course, made by side chains and/or polar atoms of the backbone. These segments contain residues highly conserved in immunoglobulin subclasses and classes. Of particular interest is the conservation of the three hydrophobic residues in C_{H2} (Leu-Met-Ile (251-253)) in all IgG subclasses, but also in IgM C_{H3} (Ile-Phe-Leu (352-354))²⁶ IgA C_{H2} (Leu-Leu-Leu (256-258))²⁷ and IgE C_{H3} (Leu-Phe-Ile (339-341))²⁸. We note in passing that this observation adds to the evidence that in IgM and IgE, which contain an additional constant domain, C_{H3} resembles C_{H2} in IgG. In C_{H1} and C_{H3} ('top' contact area) the contact residues are from the bends C-terminal to the two intra-domain Cys residues. In V_H and C_{H2} ('bottom' contact area) these are predominantly residues of the bend N-terminal to the first intra-domain Cys residue. It is of further interest that the top contact segments described for C_{H1} and C_{H3}, when projected into a V segment, coincide with the first and third hypervariable segments forming the antigen-binding surface in V dimers.

In an allosteric immunoglobulin model which we will propose in a later discussion, the signal transfer through the domains from the top contact to the bottom contact occurs along homologous segments in all domains.

The rigidity of Fab and Fc fragments and the segmental flexibility in the complete molecule Kol might just be a coincidence and future studies might provide a better statistical sample

of the whole population of molecules. The following model-building studies and discussion are based on the hypothesis that (1) rigidity and segmental flexibility are indeed inherent properties of fragments and complete, unliganded molecules, respectively, and (2) the rigid Fab fragment structures are characteristic for the liganded molecules. This is primarily based on the observation that Fab fragments show no gross structural change upon hapten binding^{22,23}. We are aware that different explanations for this observation are possible. In particular, hapten binding might be insufficient to produce structural changes brought about by antigen.

We constructed a hypothetical liganded antibody molecule by combining the fragment molecules. Starting with the Kol (Fab)₂, we set the Fab elbow angle to 120°, which is characteristic for Fab fragments. The hinge peptide, which is well defined in the Kol crystals and less well ordered—but apparently bent back—in Fc fragment crystals were superimposed with the local diads coinciding. Fc was then rotated azimuthally in order to avoid overlap between C_{H1} and C_{H2} of the same molecule. A narrow range of the azimuthal angle turned out to be possible. The resulting model is shown in Fig. 5. C_{H1} and C_{H2} are close at the bottom and top contact segments discussed before, which comprise conserved residues in immunoglobulin subclasses and classes. The hinge peptide forms numerous internal contacts with both domains and the carbohydrate. It is obvious that in view of the (Fab)₂ flexibility of the Kol molecule, the choice of the relative arrangement of the Fab arms was arbitrary. By a (Fab)₂ rearrangement the formation of a lateral contact between the Fab arms appears possible.

The important aspect of this model, which we want to emphasise, is that all longitudinal interdomain contacts are closed, resulting in a rigid structure. (Fab)₂ bending is prohibited by the C_{H1}-C_{H2} interaction.

We suggest that antigen binding causes a stiffening of the flexible antibody molecule by formation of the longitudinal inter-domain contacts described. The largest structural change involves the folding of the hinge peptide in between the C_{H2} globules to allow the C_{H1}-C_{H2} approach. This model allows deletion of a large proportion of the hinge segment as observed in two crystalline antibodies^{29,30}, without change in the relative Fab Fc arrangement, in contrast to the flexible model with the extended hinge segment as described before. The hinge deletion forces C_{H1} and C_{H2} to form the contact. This could induce an overall quaternary structural change to the rigid conformation. The Fab bending produces a model more like a T compared to the Y of the Kol molecule (Figs 3 and 5). A T-shaped model has been suggested from low resolution X-ray studies of the myeloma protein Dob³¹. This protein has a hinge deletion³². Electron micrographs of hapten cross-linked antibodies generally show a short Fc stem as we would expect from Fig. 5^{32,33}.

Segmental flexibility between Fab and Fc parts of antibody molecules is experimentally established by spectroscopic and hydrodynamic observations^{34,35}. Hydrogen-exchange experiments, as well as experiments with limited proteolysis of IgM and their Fab fragments, may be interpreted in terms of a less flexible fragment structure^{36,37}.

There are several observations of structural changes in

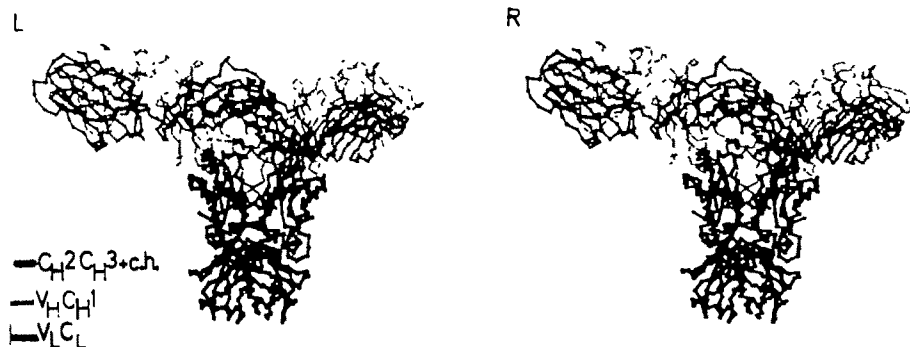


Fig. 5 Stereogram of a hypothetical liganded IgG molecule. The molecule is T-shaped with Fc forming the stem. Fc and (Fab)₂ are in close contact. The hinge peptide is folded back in between the C_{H2} parts. c h., carbohydrate.

antibody molecules upon antigen binding. Circular polarisation of fluorescence (CPL) changes in complete antibodies upon antigen binding, but not in Fab fragments^{38,39}. An antigen-induced volume contraction has been found by small angle X-ray scattering in complete antibodies, but not in Fab fragments^{40,41}. Antibodies seem to lose flexibility on antigen binding^{42,43} and to gain conformational stability against denaturation⁴⁴. The structural difference in the Fab part between complete antibody and Fab fragment ('elbow' bending) might manifest itself in a change of the characteristics of ligand binding. Observations of differences in rate constants of fragments and complete antibodies are controversial^{45,46}. There are, apparently, no significant hapten binding affinity differences between Fab fragments and complete antibodies, indicating that there is no cooperativity between the two hapten binding sites in an antibody molecule⁴⁷. This does not contradict the ligand-induced structural change proposed, as the energy required for the conformational change might be provided by an increased ligand affinity with both effects cancelling out. There are indeed observations on IgM molecules of differences in thermodynamic parameters for haptens and antigens⁴⁸, interpreted as indicating an antigen-induced structural change.

Before the X-ray structure determination of a liganded antibody molecule, there are experiments that could disprove or verify the hypothetical model of ligand induced conformational changes proposed. Chemical modification of residues participating in the longitudinal inter-domain contacts might help in determining the origin of the spectroscopic signal produced by ligand binding^{38,39}. The hinge peptide undergoes the most extensive local structural change in our model. This should be reflected in the kinetics of disulphide reduction or proteolytic cleavage. The crystal structure analysis of the Fab fragment Kol is under way in our laboratory. This molecule we would expect to be in the bent conformation. Detailed X-ray models of the IgG molecules Dob^{39,41} and Mcg^{39,49}, which have a deletion in the hinge region, would be of great interest, as they might resemble liganded molecules.

We have used the PROTEIN program system written by Dr Wolfgang Steigemann in this laboratory. We thank Drs G. Schwick and H. Haupt, Behringwerke, Marburg, for providing the Fc material, Frau K. Epp for X-ray technical assistance and the Deutsche Forschungsgemeinschaft and Sonderforschungsbereich 51 for financial assistance.

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letters to nature

Atmospheric gravity wave production for the solar eclipse of October 23, 1976

CHIMONAS and Hines¹ suggested that during a solar eclipse the cool shadow of the Moon moving with supersonic speed through the Earth's atmosphere should generate bow waves. Upper atmospheric disturbances, having quasi-periods of some 20 min, were recorded^{2,3} after the eclipse of March 7, 1970, and were regarded as possible verification of Chimonas and Hines's¹ suggestion. Other observations^{4,5} following the eclipse of June 30, 1973, failed to detect such disturbances. The eclipse of October 23, 1976, will provide another opportunity in attempting to record eclipse-generated atmospheric disturbances.

As a result of the curvature of the eclipse path and variations in speed of the Moon's shadow, focusing of the waves should occur at calculable positions and times^{6,7}. Enhanced amplitude in a focal region should provide optimal conditions for verifying the existence of the bow wave⁷. We have made calculations of the form of the bow waves expected for the eclipse of October 23, 1976, and in particular the positions and times at which focusing effects are expected, using the eclipse path calculated by Fiala and Duncombe⁸, and the technique outlined by Beer and May⁹. Figure 1 shows the calculated positions of the northwards and southwards moving bow wave-fronts, as plots of constant time, T . Ray paths lying along great circles, are also indicated for disturbances originating at time t .

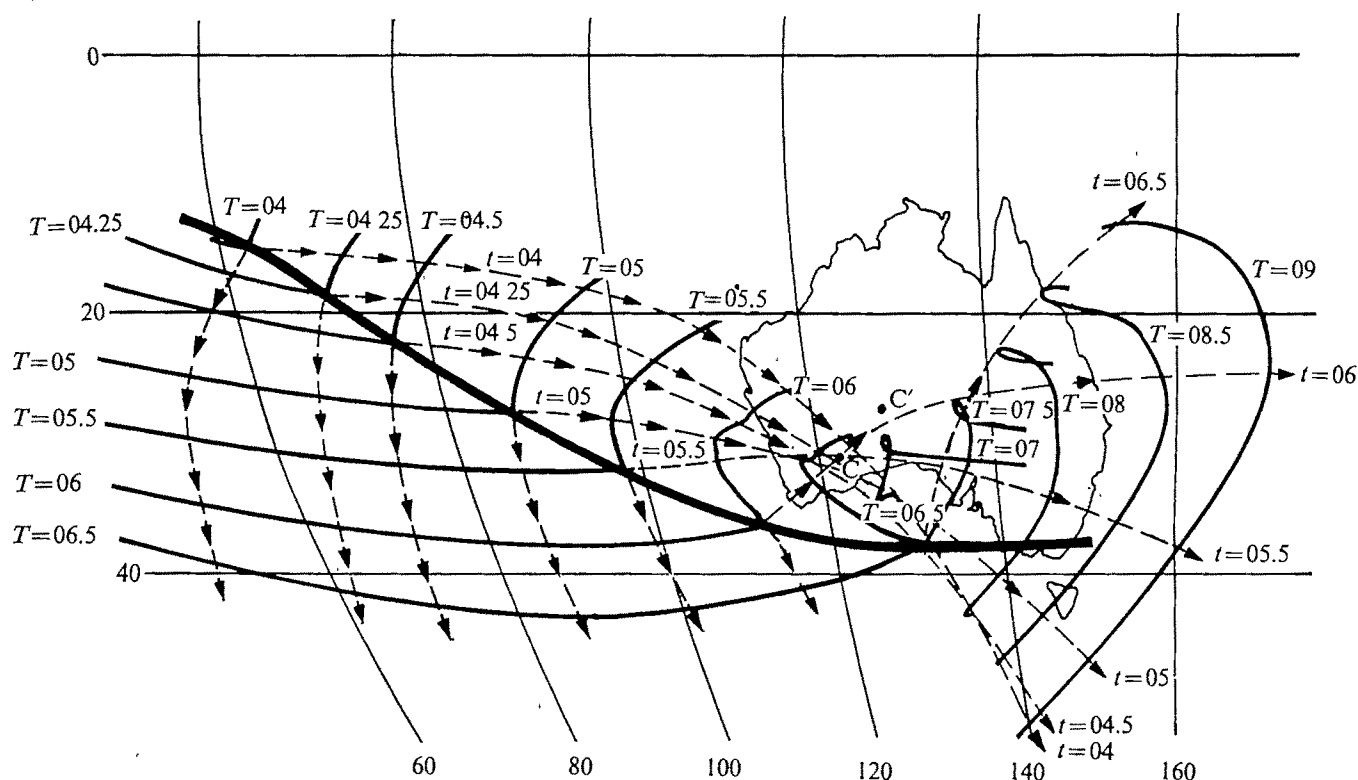


Fig. 1 Focusing of bow waves. The eclipse path is represented by a heavy continuous line. The wave fronts (continuous) are drawn for constant values of T . Ray paths (dashed) are shown for disturbances originating at times t . C_L is taken to be $10.3^\circ \text{ h}^{-1}$. When a value of 7.8° h^{-1} is assumed, the focus at C moves to C' .

The calculations were made for two values of C_L , the velocity of propagation of the bow wave, namely $10.3^\circ \text{ h}^{-1}$ (320 m s^{-1}) and 7.8° h^{-1} (290 m s^{-1}). Actual propagation velocities of long period internal gravity waves are likely to be between these values.

A well defined focus, near C in Fig. 1, appears in Western Australia where the southwards waves generated between approximately 0400 UT and 0600 UT tend to converge for $C_L = 10.3^\circ \text{ h}^{-1}$. C' in Fig. 1 indicates that focus for $C_L = 7.8^\circ \text{ h}^{-1}$.

Table 1 Approximate position and occurrence time of focus

C_L ($^\circ \text{ h}^{-1}$)	7.8	10.3
Latitude ($^\circ \text{S}$)	27	32
Longitude ($^\circ \text{E}$)	127	123
T (UT)	07.5	06.6

h^{-1} . The approximate position and T for the foci are indicated in Table 1. C and C' identify a comparatively small region where enhanced bow wave amplitude should occur.

The focus is well within the penumbra region where the bow wave contains predominantly shorter wavelength components. Quasi-periods of ~ 20 min should therefore be observed^{2,3}, rather than longer periods of some 4 h which should predominate at great distances¹⁰.

Some aspects of the discussion are over-simplified. Firstly, the eclipse path is regarded as a curved line following the path of totality, so that disturbances generated within the penumbra are ignored. Secondly, the discussion considers only geometrical optics, whereas diffraction effects will produce a more diffuse focus than indicated in Fig. 1. A disturbance period of ~ 20 min corresponds to a wavelength of 350–400 km, which will be the order of the diffraction pattern diameter.

Finally, the bow wave is assumed to propagate at constant speed along great circles, and complications arising from the structure of the atmosphere are ignored.

The recording of ground level microbarograph and ionospheric disturbances in the focal region would be most interesting.

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Argon fluorescent X rays in the Earth's atmosphere during solar flares

THE satellite Ariel V carries a proportional counter pointed along the spin axis. We report here the detection of a strong flux of X rays coming from the sunlit Earth during two large solar flares. These albedo X rays are proportional to the solar X-ray flux and consist of scattered X rays and a strong fluorescent line at 3 keV from atmospheric argon. There is also a weaker line at ~ 7 keV which is probably present in the incident solar spectrum.

The MSSL proportional counter (Experiment C) on Ariel V is a 92-cm² aperture, 85- μm thick Be window, Xe-filled counter. The field of view is circular with FWHM = 3.5° and is inclined 1.75° to the spin axis. This results in a field of view

(FOV) of 5.2° FWHM for the observations to be described. The experiment operates in one of two gain modes originally covering the energy range 1.5–20 keV.

Ariel V is in a near equatorial orbit with inclination 2.9° , apogee and perigee 543 and 501 km, and period 102 min. The satellite spins about its axis once every ~ 6 s.

During the flare of March 28, 1976, the experiment was in a low-gain multiscaler mode. The data recorded were total counts in the energy range 2.0–6.3 keV as a function of time in 64-s intervals.

During the flare of April 30, 1976, the experiment was in a spectral mode. The pulse height spectrum was recorded in 32 channels covering the energy range 1.5–9.5 keV and integrated over each orbit. Since the experiment operates only when the satellite is in sunlight, and is turned off when in the region of high charged particle flux over the South Atlantic, the average on-time is ~ 50 min. During this time the satellite views in sequence: a region of space containing the cosmic X-ray source of interest, the sunlit Earth, and the dark Earth. The sunlit Earth was a very strong X-ray source during the flare on April 30. The spectrum of the sunlit Earth was easily derived using the spectra recorded during the previous orbits (before the flare) as background.

For the flare on March 28, the spin axis was pointed at RA = 235° , dec. = $-1^\circ 6'$. No strong X-ray sources were in the FOV. The counting rate pointed at space was ~ 2 counts s^{-1} . This decreased to ~ 1 count s^{-1} when the dark Earth passed through the FOV and diffuse X rays from space were cut off. Just after the flare the rate increased to 5,000 counts s^{-1} when the sunlit Earth was viewed. Since the space rate remained at ~ 2 counts s^{-1} , there is no doubt that the sunlit Earth was the source of this signal.

The solar X-ray flux was monitored in two energy bands by NOAA ion chambers aboard the satellite GEOS 1 (ref. 1). These bands were 1–8 Å (1.5–12 keV) and 0.5–4 Å (3–24 keV). Ariel data can be normalised to the solar 1–8 Å flux or the 0.5–4 Å flux. The scattered flux seems to decay at about the same rate as the solar flux in either of these bands. We will use the 1–8 Å band because the ion chamber which produces these data has a window of about the same thickness as that of the Ariel detector. During the two flares which we observed, the solar flux in the 0.5–4 Å band was one tenth of that in the 1–8 Å band.

Figure 1 shows that the solar flux can be used to predict the X-ray strength of the sunlit Earth. When the solar flux is

Fig. 1 The solar X-ray flux in two energy ranges, (—, 0.5–4 Å; ---, 1–8 Å) recorded during the flare of March 28. The right hand scale refers to the solar flux. The scale factor for each curve has been indicated. Note that the two curves have been normalised. The short heavy lines are the normalised counting rate of the Ariel detector. These rates are given on the left hand scale and the numbers (for example, 8,051) are orbit numbers. As expected, the albedo X rays follow the measured solar flux.

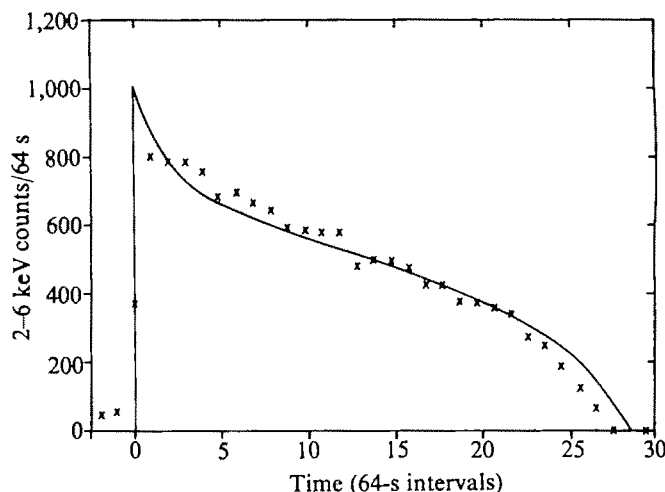
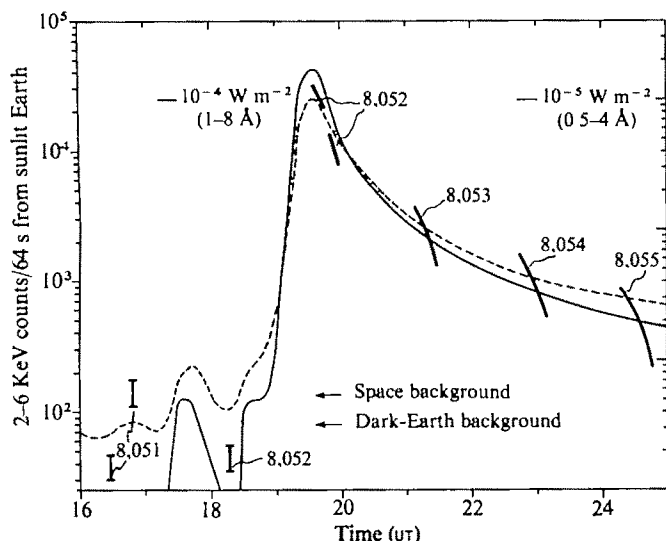


Fig. 2 The solid curve is the predicted dependence of scattered X-ray flux on scattering angle. It has been normalised to data taken during orbit 8,055 (x) to show that we understand the basic scattering geometry.

$5 \times 10^{-7} \text{ W m}^{-2}$ in the 1–8 Å band the scattered signal is expected to be about the same as the space background. Normally, when the Sun is not active, solar energy is $< 10^{-7} \text{ W m}^{-2}$ and the sunlit Earth is not a large source of background in our energy range. (This is not at all the case for softer X rays, particularly < 1 keV where fluorescent N and O lines are strong².)

Before deriving the scattered solar flux it is necessary to consider the geometry of our observation. Figure 1 shows that during each orbit the Ariel counting rate decreased much more rapidly with time than the solar X-ray flux. Since the satellite spin axis remains fixed in space, as the satellite circles the Earth it views different areas of the top of the atmosphere, and the angle that detected scattered X rays make with the terrestrial radius vector changes. The total thickness of atmosphere that these X rays must traverse, to travel from Sun to Earth to detector, also changes. Thus the detected scattered flux depends strongly on the position of the satellite in its orbit.

A simple calculation fits our data well. For these particular observations, the top of the atmosphere viewed by the detector was approximately planar, the X-ray absorption coefficient was almost the same for incident and scattered X-ray, there was only single scattering, and the cross-section did not vary much with angle (the scattering angle was always $> 90^\circ$). If the angle between the path of the incident X ray and the radius vector is ϕ , the angle between the path of the scattered X ray and the radius vector is θ , and the solar flux is f_0 , then the scattered flux at the satellite, f_s , is

$$f_s = \text{constant} \times f_0 \left\{ 1 + \frac{\cos \theta}{\cos \phi} \right\}^{-1}$$

This is shown in Fig. 2.

The agreement with our data is quite good considering that we have not smeared the prediction over the 5° detector FOV and that we have not included the time dependence of the solar flux.

The maximum flux in Fig. 2 corresponds to a time when the satellite views the sunlit limb of the Earth and the detector is looking parallel to the top at the atmosphere ($\theta = 90^\circ$). When the scattered flux drops rapidly to zero the detector is looking at the terminator ($\phi = 90^\circ$) and solar X rays are absorbed before they reach that part of the atmosphere viewed by the detector.

We note that scattering in our energy band must occur predominantly at altitudes between 70 and 90 km. At these altitudes and at sea level $\sim 1\%$ by volume of the atmosphere is argon.

We also note that Harries and Francey³ observed scattered solar X rays in the range 2–6 keV and showed that their rocket-borne detector background was consistent with this.

For the flare of April 30, spectra were recorded during orbits 8,555 and 8,556, which both showed a scattered solar continuum and a strong argon fluorescent line. We have analysed the data from orbit 8,555 which were recorded on April 30 from 2144 to 2203 UT. During this interval the solar flux averaged $2 \times 10^{-5} \text{ W m}^{-2}$ in the 1–8 Å band⁴.

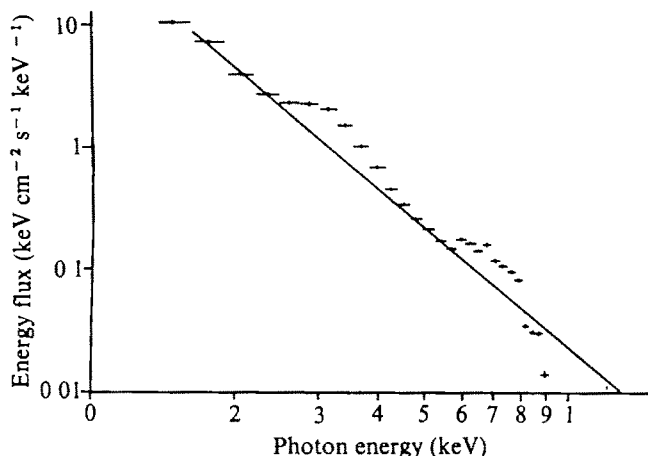
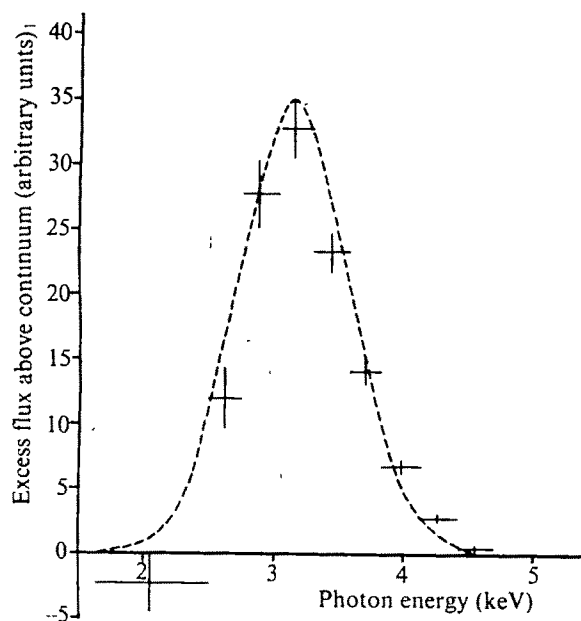


Fig. 3 X-ray energy scattered from the sunlit Earth measured during orbit 8,555. The argon fluorescent line and a scattered solar Fe XXV line(s) can be seen superimposed on the solid line which approximates the scattered solar continuum. Error bars show counting statistics only. Above 8 keV there is an additional uncertainty caused by background subtraction. The deviation of the last points from the line is not significant.

A continuum shown as a solid line in Fig. 3 was subtracted from the data to yield the line profile shown in Fig. 4. The energy and width of the line at ~3 keV are very close to the expected response of our detector to the argon line. The line is not exactly centred at the expected energy of 2.95 keV, because of the uncertainty in our detector calibration. The relative strength of the line and scattered continuum are as expected. This feature is without doubt the argon fluorescent line.

The solar spectrum during a flare consists of a continuum with strong lines due to Si XIII at 1.8 keV, Si XIV at 2.0 keV,

Fig. 4 The observed line shape compared to the expected detector response to a line at ~3 keV. Error bars are counting statistics only and do not include uncertainties in the continuum which has been subtracted.



Ca XIX at 3.9 keV and Fe XXV at 6.5 keV (ref. 5). The scattered Fe XXV feature is present in the spectrum illustrated in Fig. 3, but the other lines, if present, are hidden by the fluorescent A line at 3 keV.

We assume that the detector was looking at the Earth in the same direction as incident solar radiation ($\theta = \phi$), and also that the satellite was in a near-Earth orbit, so that the Earth can be considered as a planar source and the detector count rate is independent of altitude. The numbers following are calculated for a solar flux of $1.0 \times 10^{-4} \text{ W m}^{-2}$ in the 1–8 Å band, the maximum flux during each of the two flares we observed.

From the spectral data we derive 550 argon photons $\text{cm}^{-2} \text{ s}^{-1} \text{ ster}^{-1}$. This is approximately equal to the number of scattered photons $> 3.2 \text{ keV}$.

The total albedo energy from 1.5 to 9.0 keV (1.4–8 Å) is $1.5 \times 10^4 \text{ keV cm}^{-2} \text{ s}^{-1} \text{ ster}^{-1}$. This is equivalent to 1.5×10^{-3} of the incident solar energy re-emitted into 2π steradians. Eleven per cent of this albedo energy is in the argon line. The strength of the argon fluorescent line has been calculated by Aikin⁶ for quiet Sun conditions, but our observations do not agree—we get a factor of 100 less than Aikin.

For our detector, the rate at the peak of the flare caused by the X-ray albedo (1.5–9 keV) was 440 counts s^{-1} , 100 counts s^{-1} came from the A line and 7 counts s^{-1} from the scattered Fe line.

The X-ray flux from the Sun is normally $< 10^{-7} \text{ W m}^{-2}$ in the 1–8 Å band. Thus the normal background contribution of the sunlit Earth should be $< 0.4 \text{ counts s}^{-1}$. By way of comparison the dark-Earth background is 1 count s^{-1} and the background looking at a source-free region of space is 2 counts s^{-1} .

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Penetration of interstellar dust into the Solar System

THE Sun moves through local interstellar matter with a velocity of $\sim 20 \text{ km s}^{-1}$ (refs 1–4). Bertaux and Blamont⁴ have suggested that interstellar dust grains streaming into the Solar System are focused by the gravitational attraction of the Sun and that, as a consequence, the density of these dust grains is strongly peaked on a line extending from the Sun backwards in the downstream direction. Comparison of the predictions of this focusing model with direct observations of the dust⁵ (which do not show this large peak) leads Bertaux and Blamont to suggest that interstellar dust particles are underabundant in the Solar System by a factor of ~ 100 , compared with the usual picture of the density of interstellar grains. They suggest a number of possible explanations: first, the experiments are only sensitive to a fraction of the dust particles; second, radiation pressure or some other process eliminates the particles; third, the interstellar gas and dust near the Sun are abnormal. We point out here that a general effect not considered by Bertaux and Blamont offers a natural explanation of the observations. Grains, both in interplanetary and in interstellar space, will, in

general, carry a net electric charge, and the Lorentz force is strong enough to destroy gravitational focusing.

Several processes contribute to the charging of grains in space. The ejection of photoelectrons and the accretion of ions tends to charge the grains positively, while the accretion of electrons produces a negative charge. Since the cross section for the accretion of charged particles depends on the electrical potential of the grain⁶ the equilibrium charge state is given by a relation of the form

$$\sigma_e(V_0)f_e = \sigma_i(V_0)f_i + \gamma\sigma_{ph}f_{ph} \quad (1)$$

where the σ are the cross sections of the grains for electrons, ions and energetic photons and the f are the fluxes of electrons, ions and energetic photons; γ is the photoelectron yield (typically ~ 0.1 (ref. 7)) and V_0 is the electrical potential of the grain. Watson⁷ has computed the equilibrium electrical potential of grains in the interstellar medium to be positive, from ~ 0.1 V in cool, dense regions of interstellar space to ~ 3 V in hot tenuous regions. Grains impinging on the Solar System are thought to originate in a hot tenuous region⁴ and would thus be charged to ~ 3 V. Within the Solar System grains are subjected to large fluxes of electrons and ions from the solar wind and to energetic photons from the Sun. The photon flux is larger than the electron flux, so the grains are positively charged to a potential estimated at ~ 10 V (ref. 8). The sign of the potential is irrelevant to our argument.

A grain of radius a charged to a potential V_0 carries a net charge

$$q = aV_0/300 \text{ e.s.u.}$$

If the grains have radii⁴ of 2×10^{-6} cm and a nominal electrical potential of 5 V, then $q = z|e| \simeq 3.3 \times 10^{-8}$ e.s.u. (corresponding to a deficit of ~ 70 electrons per grain).

The Lorentz force exerted by the solar wind magnetic field on a grain having velocity V_g is

$$\mathbf{F}_L = q \left[-\frac{\mathbf{V}_{sw} \times \mathbf{B}_\perp}{c} + \frac{\mathbf{V}_g \times \mathbf{B}}{c} \right] \quad (2)$$

where \mathbf{B}_\perp is the component of the magnetic field perpendicular to the radial solar-wind velocity vector. The solar-wind velocity, V_{sw} , is ~ 400 km s⁻¹, so the initial velocity of the grain makes a negligible contribution to the Lorentz force. If we denote by \mathbf{B}_\perp^0 the transverse component of the magnetic field at the orbit of Earth and assume the field to have an Archimedian spiral structure⁹, then equation (2) can be written

$$|\mathbf{F}_L| = \frac{qV_{sw}|\mathbf{B}_\perp^0|}{c} \frac{\sin\theta}{r} \quad (3)$$

where r is expressed in astronomical units and θ is the solar colatitude angle. Then if \mathbf{F}_G denotes the gravitational attraction of the grain by the Sun

$$\frac{|\mathbf{F}_L|}{|\mathbf{F}_G|} = \frac{(2.25 \times 10^{26})qV_{sw}B_\perp^0 r \sin\theta}{GM_\odot mc} \quad (4)$$

where as above $z = |q/e| \sim 70$. Estimating the radius of the solar wind cavity to be ~ 50 AU, and taking the average value of $\sin\theta$ to be ~ 0.5 , for the grains treated in the gravitational focusing model, $F_L/F_G \sim 10$ at the orbit of Earth, and $F_L/F_G \sim 500$ in the outer reaches of the solar wind. Thus it is clear that unless the estimates of the grain charge are grossly in error, the Lorentz force dominates the motion of all but the most massive grains throughout most of the Solar System; the Sun's gravitation is a minor perturbation to the motion of small, electrically charged grains in the Solar System.

The precise effect of the Lorentz force on the trajectories of grains depends on the geometry of the interplanetary magnetic field. There is evidence that the interplanetary field has a generally uniform large scale structure¹⁰. For our purposes, it is sufficient to compare the deflection of a grain produced by the Lorentz force to that produced by the Sun's gravity. A grain moving at an average velocity of 30 km s⁻¹ spends ~ 8 yr crossing to the centre of a 50-AU solar wind cavity. In that time the Sun's gravity deflects the grain's trajectory less than a radian, corresponding to a transverse velocity increment of ~ 10 km s⁻¹. Integrating the Lorentz force (equation 2) over a particle's trajectory yields a transverse velocity increment $|\Delta V|$ about equal to the smaller of $8.6 \times 10^6 z \ln(r_0)$ cm s⁻¹ or V_{sw} , where r_0 is the radius of the solar wind cavity in astronomical units. For $z \simeq 70$ and $r_0 \simeq 50$, $\Delta V \sim 400$ km s⁻¹. This corresponds to the particle being 'picked up' by the solar wind, gyrating about the magnetic field, and being convected out of the Solar System. The precise structure of the magnetic field or the details of the grain's motion are not relevant to our main point, which is that deflection of charged grains by the Lorentz force will obliterate the gravitational focusing effect so that one should not expect to see the predicted peak.

The reader can easily convince himself that the conclusion is not strongly dependent on the parameters used in this discussion. Interstellar grains (except those having a charge to mass ratio $\leq \sim 10^{-3}$ of that presumed here) will not undergo solar-gravitational focusing and will, in fact, be largely excluded from the Solar System by the sweeping action of the solar wind magnetic field.

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Seiches in supergranules

THE recent report by Hill, Stebbins and Brown¹ of oscillations of an apparent solar radius has stirred a mild controversy². In particular, the failure of Grec and Fossat³ to detect Doppler variations with similar periods in solar spectral lines provides striking contrast with the results of Hill *et al.* Both sets of observations have been carefully performed and thoughtfully analysed and, if one accepts both sets of results at face value, the problem of reconciling the two must be confronted. Here we consider seiches in supergranules as a possible cause of the discrepancy.

Hill *et al.* in Arizona detected a discrete spectrum of oscillations in an effective diameter of the Sun, with periods decreasing from ~ 1 h. Apparent variations in this diameter can be caused by changes in the limb darkening function arising from temperature and density perturbations, but, if one assumes that the measures correspond to variations of the radius, one infers motions of the solar surface with velocities ~ 10 m s⁻¹. On the other hand Grec and Fossat in Nice have measured

the radial velocity of the solar material, averaged over the bulk of the solar surface, and find no motion with mean velocities $> 70 \text{ cm s}^{-1}$ on timescales $\sim 1 \text{ h}$ (though oscillations with a period of 5 min are clearly seen). If Hill's results arose from coherent oscillations of the solar surface it would be hard to explain the failure of the Nice group to see them, even if the Arizona results were partly a result of temperature variations.

The apparent discrepancy would be resolved if the variations detected by Hill *et al.* are the result of motions which are not coherent over the solar surface, so that they would be washed out in the Nice measurements. Indeed, the technique of Hill *et al.* would be particularly sensitive to motions with a coherence scale comparable with that over which they average.

Without going into the details of the apparatus of Hill *et al.*, we note that their slit length is $\sim 100''$, which corresponds to twice the supergranular scale of 30,000 km. Thus the results of Hill *et al.* represent an average over a few supergranules, whereas the Grec-Fossat measurements average over $\sim 1,000$. Appropriate motion in the supergranules could therefore explain the difference in results.

Supergranule motions are seen in the solar surface and look like convective cells, with upwelling in the centres and with horizontal outflow over most of the surfaces at speeds of $\sim 0.5 \text{ km s}^{-1}$ (refs 4, 5). These motions are distributed over the solar surface in a roughly polygonal network. At parts of the interface between the supergranules, vertical magnetic fields are observed with intensities of $\sim 1,800 \text{ Gauss}$ (ref. 6). These fields are concentrated in small spots which are located preferentially at the corners of the supergranules. Unfortunately, we have no direct knowledge of the field topology below the visible layers of the Sun. On theoretical grounds it is believed that these fields blend into a prevalent toroidal field well below the surface. Whether the observed flux ropes merge in relatively shallow layers or remain discrete over great depths is, however, not known.

Let us suppose the former possibility. Then the lower parts of the supergranules are partly separated by the magnetic field. Thus, we may consider the fluid in each supergranule to be loosely contained in a roughly fashioned, rather leaky cup. The cup walls would of course be flaccid, since the fields would probably be much weaker than the values observed in the small dots seen in the surface layers.

We propose that within these magnetic cups there occur seiches (slow sloshing motions). Such motion is well known to limnologists and is readily observed in a mug of beer held in an unsteady hand. The motion has little shear, and should therefore have low dissipation except in the very turbulent upper layer. At the speed suggested by the observations of Hill *et al.*, the supergranular seiches should be nearly incompressible. If we presume that the depth of a cup, h , is $\sim l$, the supergranular diameter, then the scale height near the bottom is $\sim h$; only in the upper layers do we face great inhomogeneity. We may therefore estimate the period P of the fundamental seiche mode using Merian's formula⁷

$$P = \frac{2l}{(gh)^{\frac{1}{2}}} = 11 \left(\frac{l}{h} \right)^{\frac{1}{2}} \text{ min}$$

where g is the solar surface gravity. Of course this is a very crude estimate, since a number of complicating factors have been ignored. In particular, the restraining effect of the magnetic field has probably been grossly exaggerated, and it is likely that the period should be considerably longer than this formula suggests. Nevertheless, Merian's formula gives some idea of the order of magnitude of the period if we know the value of l/h .

It has been suggested⁸ that supergranules are squat with $l/h \simeq 5$. The arguments for this are not compelling, and in fact we do not know why there should be such a distinct cell size in what appears to be a very turbulent layer. Moreover, we have to distinguish between the depth to which traces of the horizontal pattern persist and the depth we would attribute to a coherent

motion of the sort we are contemplating. The latter depth, which is h in our picture, is that at which the toroidal magnetic field becomes appreciable and is able to provide effective basins in which the supergranules may rest. If we suppose that the surface magnetic field is anchored in this toroidal field we can estimate this depth.

One might suppose that the depth dependence of the solar angular velocity Ω near the equator is such that the differential rotation maintains approximate neutrality according to Rayleigh's criterion. Then near the equator

$$\Omega \simeq \Omega_s \left(\frac{R}{r} \right)^2$$

where Ω_s is the surface angular velocity, R is the solar radius, and r is the radial co-ordinate. The value of Ω at the depth of interest is reflected by that of the sunspots and the magnetic network as seen at the surface. The difference between Ω and Ω_s is $\sim 5\%$ (ref. 4). This suggests depths of $\sim 2 \times 10^4 \text{ km}$. A similar argument has been advanced by Foukal and Jokipii⁹.

Thus we have estimates for l/h in the range 1–5 and Merian's formula suggests periods in the range of 10–30 min. The flexibility of the walls will raise these values, and periods $\sim 1 \text{ h}$ seem possible.

The way in which seicheing of supergranules could affect the results of the Arizona group is complicated. Slight tilting in a suitable direction of a supergranule at the limb could modify the local limb darkening, to which the Arizona observations are very sensitive, but it seems premature to enter into such details in this very tentative model. We note that the kind of effect we allude to here gives a change in the apparent position of the limb. It may also give some change in local intensity, but if this is appreciable, the picture we propose would appear to be ruled out by recent work of Musman and Nye who have failed to detect brightness fluctuations on the solar disk with periods in the range of 15–90 min. To illustrate the kind of effect we envisage, it may be adequate to adopt the simplistic view that seiches would make their presence known through slight local elevations of the 'solar surface'.

If speeds in the seiche mode are $\sim 10 \text{ m s}^{-1}$, the picture is compatible with the observations of Hill *et al.* and also predicts that the observations made in Nice would provide negative results unless the sensitivity were increased to detect velocities significantly $< 1 \text{ m s}^{-1}$ or unless the area averaged over were appreciably reduced. Moreover, the energy in the motion is much less than that required if the observations are to be explained in terms of nearly pure global normal modes, since the seiches are probably not very deep. This is satisfying in view of the recent computations by Goldreich and Keeley¹⁰, which indicate that it might be difficult to excite such modes in the Sun by turbulent driving. As to the excitation of the seiches, various possibilities present themselves. For example, supergranules last $\sim 40 \text{ h}$, according to Simon and Worden, and seiches would probably be excited as part of the formation process. In addition, undulations of the deep toroidal magnetic field would provide rocking of the magnetic cups, and consequent excitation of the modes.

The idea of sloshing supergranules is based on assumptions about the subsurface field topology. Where the surface field emanates from the underlying toroidal field there should be significant deformations, perhaps ridges, in the toroidal field. But it may be that these structures lie too deep to confine the supergranules. Nevertheless, individual motions of supergranules might arise, though the existence of an adequate excitation mechanism is then less apparent. Among the possibilities for exploration is the collective interaction of supergranules, as also noted by N. O. Weiss. This might produce suitable surface undulations, but it is not clear what the period would be. This would also provide some degree of phase coherence. Surface waves travelling at the local sound or gravity wave speeds do, however, traverse a supergranule in $\sim 1 \text{ h}$ and long waves of this kind would have nodes at the super-

We believe, therefore, that there are sufficient grounds for suggesting that one should study motions of supergranules as individual entities. The amplitude of the motion needed to resolve the apparent conflict in the present observations suggests that the seiches would be detectable. Indeed, there are observed variations in the solar surface which indicate that such motions may actually exist¹¹.

Note added in proof: Hill¹⁴ has recently found evidence for phase coherence of the 45-min oscillation over 30 d and Kotov *et al.*¹⁵ have found phase coherence of the 2 h 40-min oscillation over 3 yr. Thus although supergranular seiching and the passage of supergranules across the field of view may contaminate the measurements, the above evidence would imply that the oscillation data cannot be attributed entirely to these phenomena.

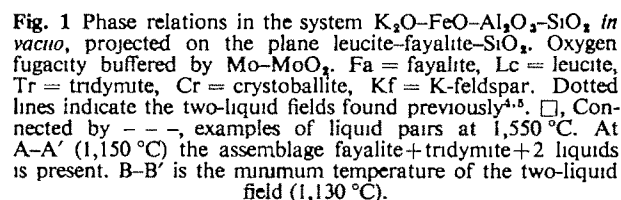
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DIFFERENTIATION in silicate magmas is usually considered to occur through processes of fractional crystallisation. The possibility that the formation of a second silicate liquid, immiscible with the original silicate magma, may take place has been thought unlikely¹. But in lunar rocks textures were found indicating late-stage formation of a K₂O-rich silicate liquid², and serious consideration is being given to the possible importance of silicate liquid immiscibility³. Current investigations on the role of silicate liquid immiscibility were started with the work of refs 4 and 5. Because of the importance of the two-liquid field in the current developments we have re-investigated the K₂O-FeO-Al₂O₃-SiO₂ system. Electron scanning microscopy combined with electron microprobe analyses allowed us to delineate the extent of the two-liquid field as well as to determine the compositions of the two coexisting liquids.

buffer, which lies within the FeO stability field⁸. Runs were made at temperatures between 1,100 and 1,700 °C *in vacuo*. The samples were kept in molybdenum capsules.

The most interesting result of our study concerns the compositional range of the two-liquid field. It extends from the binary join FeO-SiO₂ to the stability field of leucite, (Fig. 1) and incorporates the two smaller separated fields found previously. This result is based on more than 150 runs at various temperatures. Runs with initial composition in between the two miscibility gaps found previously also produced two liquids. As an example two runs at 1,550 °C are shown in Fig. 1. Electron microprobe data show that the liquids are homogeneous, indicating that equilibrium was obtained. The co-existing liquids can be characterised as a FeO-rich liquid with a K₂O/Al₂O₃ ratio of ~ 0.6 and a SiO₂-rich liquid with a K₂O/Al₂O₃ ratio of ~ 1.



The run products show that the two liquids form droplets within each other, which has been considered as the classical proof for liquid immiscibility⁴. Figure 2a shows a typical example of textural features which were found in runs made at temperatures $< 1,250^{\circ}\text{C}$ and $> 1,400^{\circ}\text{C}$, the size of the droplets varies from 3 mm to submicroscopic. In runs at intermediate temperatures separation of the two melts occurs on a submicroscopic scale only. Examples of this type of separation are shown in a series of scanning electron micrographs, see Fig. 2 If the etched glass surface did not show a texture

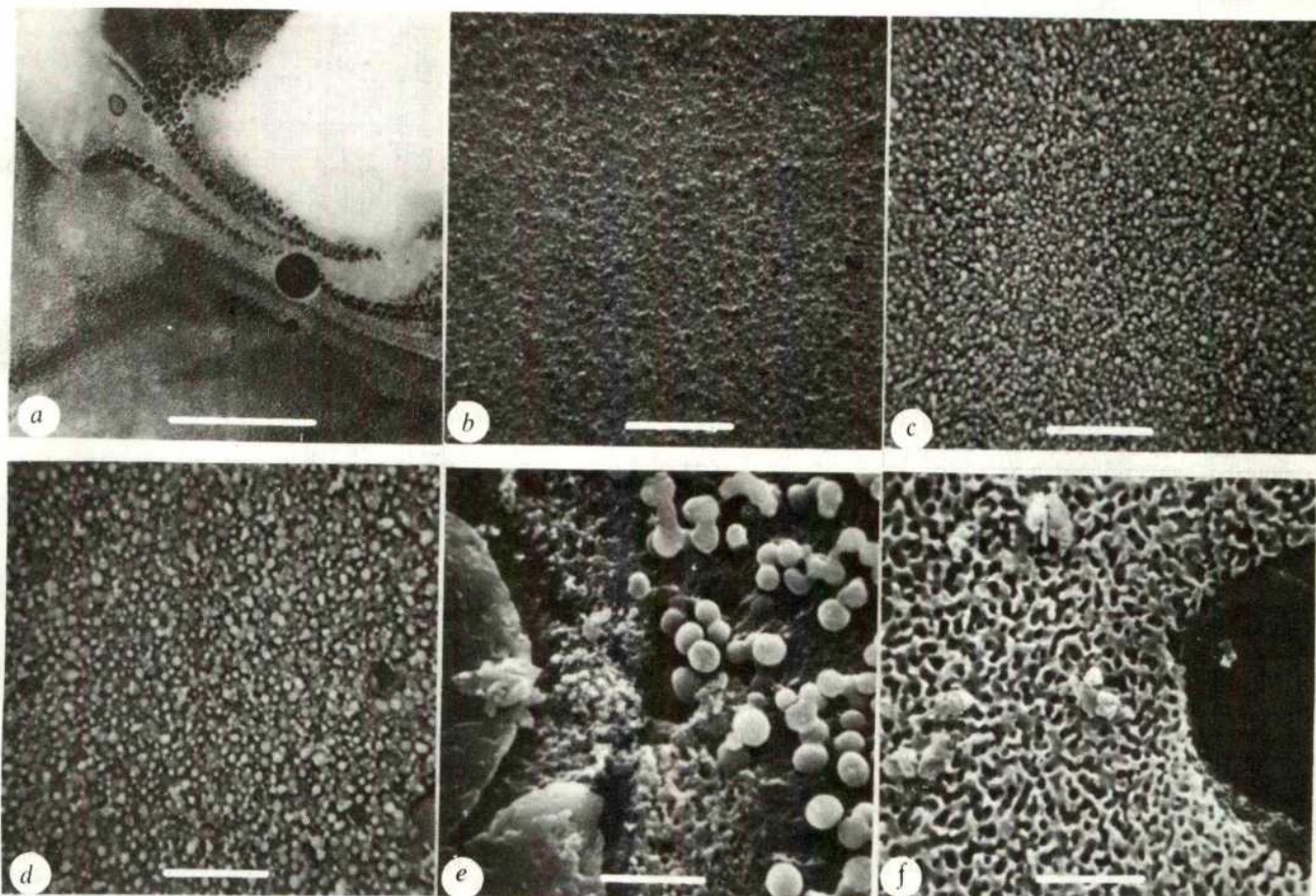


Fig. 2 Photographs of run products. Initial compositions lie roughly on the 30% FeO isopleth. *a*, Thin section of a typical run at low temperature (1,150 °C) in the middle of the two liquid field. The bar is 200 μ m long. Black spheres of a FeO-rich liquid in a matrix of colourless SiO₂-rich liquid. Note the variation in size of the spheres; grey cloudy areas (upper left) indicate submicroscopic scale separation. Pictures *b-f* are scanning electron micrographs of etched surfaces of run products. The etching agent was HF which preferentially removed the SiO₂-rich liquid. The bar is again 2- μ m long. *b*, Sample from one liquid field. Run temperature 1,300 °C (normal image). *c*, Sample from two-liquid field, just inside the two-liquid/one-liquid phase boundary. Run temperature 1,300 °C. White droplets are the SiO₂-rich glass (inverted image). *d*, Sample from the middle of the two-liquid field. Run temperature 1,300 °C. Size of the droplets is larger than in *c* (inverted image). *e*, Sample from the two-liquid field, close to the FeO-SiO₂ join. Run temperature 1,300 °C. Spheres are the FeO-rich liquid. On the left hand is a tridymite crystal (normal image). *f*, Sample from the leucite + two liquid field. Run temperature 1,200 °C. Black spherical cavities indicate the SiO₂-rich glass removed by etching. On the right side is a leucite crystal (normal image).

indicating unmixing (Fig. 2*b*) the liquid was considered to be homogeneous. If droplets of one liquid were found in a matrix of another, clearly two liquids were present, and the run was considered to lie within the two-liquid field (Fig. 2*c-e*). These textures are typical for a spinodal phase separation mechanism⁷. The size of the droplets increases toward the FeO-SiO₂ join. Close to this join tridymite or cristoballite was found together with the two liquids (Fig. 2*e*). An example of leucite plus two liquids is shown in Fig. 2*f*.

The phase relations of the liquidus surface are shown in Fig. 1. The two-liquid field intersects the liquidus surface at 1,695 °C on the FeO-SiO₂ join. It extends into the system and reaches a minimum at 1,130 °C (B-B') where FeO-rich liquid reacts to form fayalite+leucite+SiO₂-rich liquid. Thus, any Fe₂SiO₇-rich liquid must produce SiO₂-rich liquids during the course of crystallisation, without the formation of liquids of intermediate compositions. An important reaction occurs at 1,150 °C (A-A'). At this temperature tridymite reacts with the FeO-rich liquid to form fayalite plus SiO₂-rich liquid. Fayalite coexists with the SiO₂-rich liquid down to the eutectic.

The geological importance of these new data is that at least for dry magmas an FeO-rich basalt must yield an immiscible granitic liquid during the later stages of

crystallisation. Liquids of intermediate composition are not formed in this process. The immiscibility textures in lunar rocks² are an example of this. On Earth, excellent immiscibility textures were found in Archean variolitic pillow lavas near Noranda (Quebec, Canada) by Gelinás *et al.*⁸. In conclusion, the suggestion that the granophyres found in close association with ferro-gabbroic rocks may be produced by a process of liquid immiscibility⁹, appears to be valid.

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Phosgene in the ambient air

IN recent years the atmosphere has become contaminated with a large number of halogenated compounds. Whereas inert fluorocarbons are suspect as precursors of stratospheric ozone-destroying chlorine atoms, other halocarbons, such as vinyl chloride, chloroform and trichloroethylene, have been found to be carcinogens^{1,2}. We present here the first measurements of ambient phosgene (COCl_2), and demonstrate that chloroethylenes (primarily C_2Cl_4 and C_2HCl_3), which are emitted worldwide in extremely large quantities (1.5×10^6 t in 1975), photodecompose to form highly toxic species such as phosgene and chloroacetylchlorides. A consideration of the sources, the distribution, and the fate of these species suggests that a significant environmental impact is possible. The results presented here are based on laboratory and field studies conducted within California in 1976.

Phosgene is manufactured primarily for captive use, and its production in the United States in 1975 was in excess of 0.4×10^6 t. It is a highly toxic chemical, although very little is known about the health hazards associated with long term exposure to low concentrations of the gas³.

A special purpose electron-capture (EC) gas chromatograph (GC) equipped with two EC detectors in series was used for ambient phosgene analysis (Fig. 1). Calibrations were performed using a permeation tube and further checked in the field using the absolute pulse flow coulometric (PFC) method developed by Singh *et al.*⁴.

The phosgene was identified by comparing three independent properties, namely, GC retention data, the EC ionisation efficiency (Fig. 1), and phosgene destruction rates on glass surfaces with standard phosgene mixtures. The possibility of interference from other trace atmospheric halogenated species (such as CCl_3F , CCl_2F_2 , $\text{CCl}_3\text{FCClF}_2$, $\text{CClF}_2\text{CClF}_2$, CHCl_2F , CHClF_2 , CH_3Cl , CH_3I , CH_3Br , CH_2Cl_2 , CHCl_3 , CH_3CCl_3 , CCl_4 , C_2HCl_3 , C_2Cl_4 , $\text{CH}_2\text{BrCH}_2\text{Br}$, and SF_6) was eliminated by retention data measurements. The high ionisation efficiency of phosgene further precludes any possible interference, since only a few known species (such as CCl_4 , SF_6) are as good absorbers of electrons. An Ascarite trap in front of the GC column was able to remove phosgene as expected. A GC-MS confirmatory test was not possible because of the high liquid phase loading of the GC column and the unavailability of other acceptable column packings.

Field monitoring was conducted on a 24 h-a-day basis at four Californian sites (urban and non-urban) with the help of a mobile environmental laboratory. Table 1 provides the results of phosgene concentrations measured at these four sites, together with the concentrations of C_2Cl_4 and C_2HCl_3 , its suspected precursors. The range of phosgene concentrations

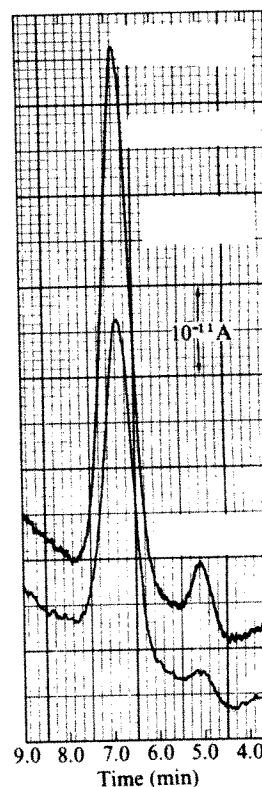


Fig. 1 Dual EC-GC chromatogram showing ambient phosgene elution for a 13.0-ml sample from Menlo Park. The separation was accomplished on a dual EC-GC, equipped with an aluminium column ($5' \times 0.25''$) packed with 30% didecyl phthalate on 100/120 mesh acid-washed Chromosorb P. This GC column was preconditioned with twenty, 5-ml injections of a 5 p.p.m. (10^{-6} v/v) mixture of phosgene and trichloroacetyl chloride in air. Both the GC column and the dual EC detector unit were maintained at 22°C . Ultrapure nitrogen (carrier gas) was passed through a sequence of traps containing charcoal, anhydrous calcium sulphate, and molecular sieve before entering the GC at a constant flow rate of 90 ml min^{-1} . All-glass syringes were used for sample injection, and the sample holdup time was kept < 15 s. The large peak is for fluorocarbon 11, retention time 6.9 min, p (ionisation efficiency) = 0.40; the small one is for phosgene, retention time 5.1 min, $p = 0.65$. The top curve is for ECD1, the bottom for ECD2, and the ionisation efficiency is given by $p = 1 - (\text{ECD2 signal/ECD1 signal})$.

was 13 to 61 p.p.b. (10^{-12} v/v) with a mean value of 21.6 p.p.b. at the remote site 3 which is $\sim 2/3$ the mean value of 31.7 p.p.b. in urban Los Angeles. This factor of 1.5 for the phosgene gradient between an urban and a remote site can be compared with a factor of 20 for its precursors. This primarily arises

Table 1 Average phosgene and precursor levels in California

Site no.	Location	Nature of site	Monitoring* period (1976)	Phosgene	Concentrations (10^{-12} v/v)	
					C_2Cl_4	C_2HCl_3
1	Lat. $34^\circ 04'$ Long. $118^\circ 11'$	Urban (Los Angeles)	28/4-4/5	31.7† (8.3)‡ Max. 61.1§	673.3 (496.8)	310.8 (301.6)
2	Lat. $33^\circ 48'$ Long. $116^\circ 33'$	Downwind of urban Los Angeles (Palm Springs)	5/5-11/5	29.3 (6.2) Max. 44.4	278.1 (232.7)	39.7 (83.6)
3	Lat. $37^\circ 39'$ Long. $119^\circ 40'$	Remote—High altitude (Badger Pass— elevation 7,800 feet)	12/5-17/5	21.6 (5.1) Max. 28.8	30.7 (10.5)	15.6 (2.5)
4	Lat. $37^\circ 24'$ Long. $112^\circ 12'$	Urban-suburban (Menlo Park)	23/5-27/5	30.3 (3.2) Max. 38.8	201.9 (413.9)	113.5 (528.5)

*All monitoring was conducted on a 24-h basis.

†The quantity before the parentheses is the mean of the entire data base.

‡The quantity in parentheses is the s.d.

§Maximum phosgene concentration.

because the precursors come from urban sources and are well dispersed by the time a significant decomposition to phosgene takes place.

The three readily identifiable sources of phosgene in air are: (1) direct emissions of phosgene during manufacture, handling, and use; (2) thermal decomposition of chlorinated hydrocarbons, and (3) photo-oxidation of chloroethylenes in the air. There are indications that the first two sources can result in a significant indoor hazard, but their contribution to the ambient budget is minimal. The photo-oxidation of chloroethylenes (C_2Cl_4 and C_2HCl_3) seems to be the most probable source of phosgene. Based on estimates from most recent laboratory studies⁵, present C_2Cl_4 and C_2HCl_3 emissions could result in the formation of $\sim 0.3 \times 10^6$ t of phosgene yr^{-1} . Because these solvents are typically emitted in urban areas and are relatively reactive (the tropospheric half lives of C_2HCl_3 and C_2Cl_4 are < 2 and 4 d, respectively), high concentrations of phosgene could be encountered during adverse meteorological conditions in and around urban centres.

The atmospheric sinks of phosgene are a matter of considerable uncertainty. The known absorption cross section of phosgene, and smog-chamber studies (where phosgene was found to be stable for 15 h under simulated tropospheric irradiations in the presence of 10,000 p.p.m. of water vapour) suggest negligible tropospheric loss through photolysis and gas-phase hydrolysis^{4,5}. The two important sinks of phosgene are heterogeneous decomposition and slow liquid-phase hydrolysis. The first sink is confirmed by the fact that phosgene at low concentrations was destroyed in contact with most surfaces within a few hours, and additionally, could not be measured in indoor spaces. During one day of field experiments, heavy overnight rain caused a 20% reduction in average phosgene concentrations. Slow dissolution in the ocean followed by hydrolysis is probably a significant sink of ambient phosgene. Other gas-phase reactions involving O and OH radicals are known to be very slow. It seems clear that phosgene is only removed slowly from the atmosphere.

Laboratory studies also suggest that the highly toxic di- and trichloroacetyl chlorides that are also formed from the photo-oxidation of C_2HCl_3 and C_2Cl_4 , respectively, would together be present at concentrations of about four times that of phosgene⁶. Methods for the measurement of chloroacetyl chlorides in the 10^{-1} – $1,000$ p.p.b. (10^{-12} v/v) range are not yet available. Secondary reactions of phosgene and chloroacetyl chlorides with ambient hydrocarbons and the heterogeneous decomposition of chloroacetyl chlorides to form chloromethanes have already been suggested⁶. It is also possible that cigarette smokers may be exposed to an additional dose of phosgene, especially in urban locations, since chloroethylenes are thermally decomposed to phosgene, and are often present at concentrations of up to 200 times the ambient phosgene concentrations.

Because of the high toxicity of phosgene and chloroacetyl chlorides, and the suggested carcinogenicity of C_2HCl_3 and its structural similarity to C_2Cl_4 , the continuous release of these chloroethylenes in such large quantities to the atmosphere may be undesirable.

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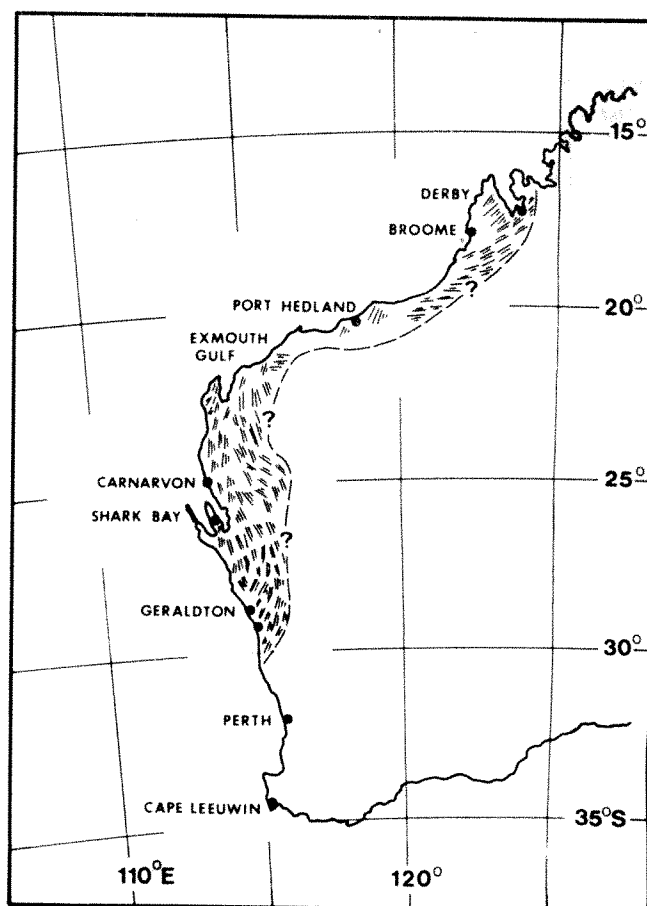
Widespread late Quaternary aridity in Western Australia

UNTIL recently the only conclusive evidence of extended arid conditions during the late Wisconsin over the western part of the Australian continent, was largely provided by the submerged dunes of the Fitzroy estuary^{1,2}. This evidence has been used to support the concept of global tropical aridity during the late Quaternary³. From a study of the late Quaternary sediments of the coastal areas of Western Australia, however, it has become apparent that stable dune fields and sand sheets of late Quaternary age are found throughout this region (Fig. 1).

It is evident from electron microscope studies of quartz grain surface textures, and from the grain-size distribution and mineralogy of the sands, as well as from the field occurrence and form of the sand deposits, that these deposits must be aeolian in origin, and derived from inland sources. In the Fitzroy estuary Holocene marine sediments overlie dunes, and give these dunes a minimum age of 7,400 b.p. (ref. 2). Linear dunes in the Exmouth Gulf have been truncated by the same transgression. Further south, a minimum age is more difficult to establish. In the Geraldton area, an inset valley fill dated at $3,145 \pm 145$ b.p., consisting largely of reworked aeolian sands indicates that arid conditions, in that area, must have ceased by that time.

A likely maximum age for the aeolian deposits can be established from the age of red alluvial sediments from which some aeolian material was deflated. In the Geraldton region the red alluvial fill overlies deposits of the Sangamon high sea level of $\sim 120 \times 10^3$ b.p. (ref. 4). Carbonaceous

Fig. 1 The recognised extent of wind-blown sand deposits in the coastal areas of Western Australia.



material from the red alluvial fill gave a ^{14}C age of $> 37 \times 10^3$ yr. The considerable thickness of the red alluvial sediments in coastal sections demonstrates that they were deposited at a time when the sea level was considerably lower than at present. An early Wisconsin age seems plausible for the red alluvium, and hence it follows that this is the maximum age of the aeolian sands.

The stratigraphy of the deposits gives no additional evidence of the precise age of the wind-blown sands. Pedocalcic palaeosols from the Timor Shelf, dated at 17,000 b.p. (ref. 5), and lunette deposits in the South-west of Western Australia, with a corresponding age of $\sim 17,000$ b.p. (ref. 6), indicate, however, that at that time arid conditions prevailed both in the North and South of the state. This provides a possible age for the aeolian sands. More important than this is the fact that the combined evidence suggests the possibility that most of the western part of the Australian continent may have been intensely arid at that time.

Although the climatic conditions necessary to bring about widespread and intense aridity over most of Western Australia would require a fundamental alteration of the present climatic regime, the actual mechanisms which can bring about such conditions require only relatively minor changes in the present heat balance and circulation pattern of the continent. The present climate of Western Australia is dominated by the subsiding air of the sub-tropical high pressure belts⁷. The seasonal displacement of this belt brings about the two distinct precipitation regimes: summer rainfall in the North, through the incursion of moist tropical air; winter rainfall in the South, from mid-latitude depressions.

The travelling anticyclonic cells within the sub-tropical high pressure belt control both precipitation regimes. In winter this is especially so. In these months the anticyclonic cells become intense, enhanced by low surface temperatures, and move slowly⁸. In these conditions the anticyclonic cells can dominate much of the continent, and in Western Australia limit rainfall to the southern shores. In some instances a large anticyclonic cell may produce a blocking effect, deflecting mid-latitude fronts approaching the West Australian coast southwards, and restricting precipitation to the south-western corner.

Even during the summer months, the anticyclonic cells, which are now situated much further South, have a profound influence on the summer precipitation regime of the northern areas. Often, when an intense surface anticyclonic cell is present in the Australian Bight, stable conditions prevail in the lower troposphere over northern Western Australia. This stability occurs in spite of the presence of a shallow hot surface region low over the Pilbara Region. Only when the anticyclonic cell moves eastwards, or decays, will the stable conditions over the northern areas weaken, resulting in an increased likelihood of rainfall from monsoonal depressions and disturbances^{9,10}.

It is evident from the present climatic situation over the Australian continent that an increase in anticyclonicity would directly result in widespread aridity over most of the western portion of the continent. The lower global temperatures which prevailed at the time of the last glacial maximum would have been sufficient to bring about an increase in anticyclonicity. With a lowering of the July temperature by $\sim 5^\circ\text{C}$ (refs. 11, 12), the winter anticyclone would have become much more stable, and the frequency of breakdown of anticyclonic cells would have been considerably lower. As a result winter rainfall would have been confined to the extreme south-west with only occasional widespread rains over the southern half of the state.

During the summer months anticyclonic conditions would have been weakened but this may have been only to such

a degree that the mean synoptic conditions would have been similar to the present-day late spring or early autumn. In addition, because of a more restricted seasonal migration of the inter-tropical convergence zone, the summer anticyclonic cells would have been located further north than at present. These factors, combined with weaker Trade winds, would have resulted in extremely stable conditions, curtailing the amount and frequency of summer rainfall over the northern areas.

An additional factor which must be considered is that cooler sea surface temperatures may have greatly reduced the significance of the offshore waters of northern Australia as a tropical cyclogenetic area¹³. Tropical cyclones contribute a considerable proportion of the present total precipitation of the northern areas of the state (for example, at Port Hedland this accounts for 50% of the mean annual precipitation). A decrease in the frequency of cyclones, whether through reduced cyclogenesis or a more westerly sea path because of stable conditions prevailing over the continent, would drastically alter the precipitation amounts in the northern half of the state.

The circulation changes necessary to result in aridity prevailing over most of the western parts of the Australian continent are physically plausible from what is presently known of global climatic conditions at the last glacial maximum. With the changes in the circulation patterns that have been proposed to explain the geomorphological evidence, it is difficult to envisage that arid conditions could have prevailed in the tropics without intense aridity further south. This reconstruction also implies that arid conditions prevailed through much of the glacial periods, becoming especially pronounced at times of glacial maxima. Even today Western Australia is very susceptible to the onset of arid conditions, a present drop of 120 mm in the total annual rainfall would extend the present margins of aridity by some $473 \times 10^3 \text{ km}^2$ (ref. 14). It follows that the envisaged circulation conditions prevailing at the last glacial maximum would have had their most obvious manifestations in Western Australia. While this is only to be expected, the increase in anticyclonicity would have had an effect on the whole continent, to such an extent that these conditions are likely to have been the determinant of Australian climatic conditions through the glacial periods.

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Evolution of ocean climate and the record of planktonic foraminifera

THE planktonic foraminiferal fossil record reveals two major evolutionary radiations, one in the Palaeogene and another in the Neogene. Preceding each radiation, at the beginning and middle of the Cainozoic, there were severe reductions in faunal diversity. Both reductions involved an elimination of tropical and sub-tropical forms and the radiations involved their restoration¹.

During the late Cretaceous and the Palaeogene (until about the Middle Eocene) faunas were diverse and well differentiated. Douglas² has shown that the late Cretaceous faunas, like modern ones, can be divided into cold- and warm-water groups (Boreal and Tethyan) and recently Szczuchura and Pozaryska³ have shown that the same is true for Palaeogene faunas. Both late Cretaceous and Palaeogene faunas, however, indicate a much greater expanse of warm water than exists today⁴.

In contrast, Danian and Oligocene faunas universally have a 'cold water' aspect. They are completely lacking in warm water morphotypes and diversities are consistently low. If subtropical or tropical pelagic habitats existed at the beginning or the middle of the Cainozoic, planktonic foraminifera give no clue to their whereabouts. Isotopic evidence indicates that the Oligocene was a cool period, possibly with glaciation at high latitudes⁵. More than that, however, the ungrouped faunas suggest rather structureless oceans that were not only cool, but lacking in the kind of climatic differentiation that exists now.

The Neogene radiation started at the beginning of the Miocene and by Middle Miocene there was a strong development of warm water forms. These forms, moreover, were widely distributed and, as in the Cretaceous and early Palaeogene, indicate a much greater expanse of warm water than exists today. The transition from Oligocene to Miocene cannot be dealt with now because of complex boundary questions. It is possible, however, to gain some idea of how modern plankton biogeography developed in the North Atlantic once warm water forms became re-established, by making faunal comparisons among several key areas around the North Atlantic Gyre.

The Mediterranean is a key area because it is stocked entirely from the eastern margin of the North Atlantic Gyre and its entrance is well north of the limits of the present Tropics. Since species are displaced to the south on the Ocean's eastern side, the Mediterranean is stocked with northern and subtropical forms but no tropical ones. The rare tropical forms that are found in the Mediterranean from Pliocene to Recent are not part of the indigenous fauna. On the western side of the ocean, displacement is to the north and tropical species are found as far north as 42°N, in the vicinity of the Gulf Stream⁶. Tropical forms are, however, abundantly represented in the Miocene Mediterranean, in particular, *Globorotalia menardii*, a modern tropical species with a geological record dating back to the Miocene. From its evolution in the Middle Miocene until the end of the Miocene, this species is common and continuously distributed throughout the Mediterranean and there can be no doubt that it was part of the indigenous fauna. It would seem therefore, that the Tropics extended much further north during the Miocene, at least to the vicinity of the entrance to the Mediterranean (36°N).

An alternative theory is a Miocene Tethyan seaway, with penetration of tropical forms from the Indo-Pacific. There is, indeed, much about Miocene planktonic faunas to suggest Tethyan relationships, but evidence from marine invertebrates seems to demonstrate convincingly that the eastern gateway to the Mediterranean was closed by about the middle of the Cainozoic^{7,8}.

Unfortunately, planktonic faunas from the western side of the North Atlantic are poorly known except for the tropical region where, of course, *G. menardii* is abundantly represented. The Deep Sea Drilling Project (DSDP) has, however, cored two

high latitude sites in the North Atlantic that have very important biogeographic implications. One site is on the western side of the ocean near Labrador (50°25'N), the other on the eastern side in the Hatton-Rockall Basin near the British Isles at 57°30' (ref. 9). The latter site yielded good late Miocene faunal assemblages that included *G. menardii*. Associated with *G. menardii* were the modern cold-water species *Globigerina bulloides* and *G. pachyderma* together with modern warm water, although not strictly tropical, species *Globigerinoides ruber*, *G. trilobus* and *Orbulina universa*¹⁰. This kind of mixed association is indicative of waters near the margin of the Gyre. The western drilling site near Labrador yielded a poor Miocene section, but faunal assemblages yielded warm-water forms¹¹. Also, early Miocene assemblages with close Caribbean affinities have been encountered near Nova Scotia¹².

These data, although sparsely distributed, indicate an expanded body of warm gyral water for the Miocene, the gyral limits extending perhaps 10° further north than they do now. Tropical limits probably also extended perhaps 10° further north than at present. This expanded warm gyral water persisted until the end of the Miocene, so it is unlikely that modern glaciation in the Northern Hemisphere began before the Pliocene.

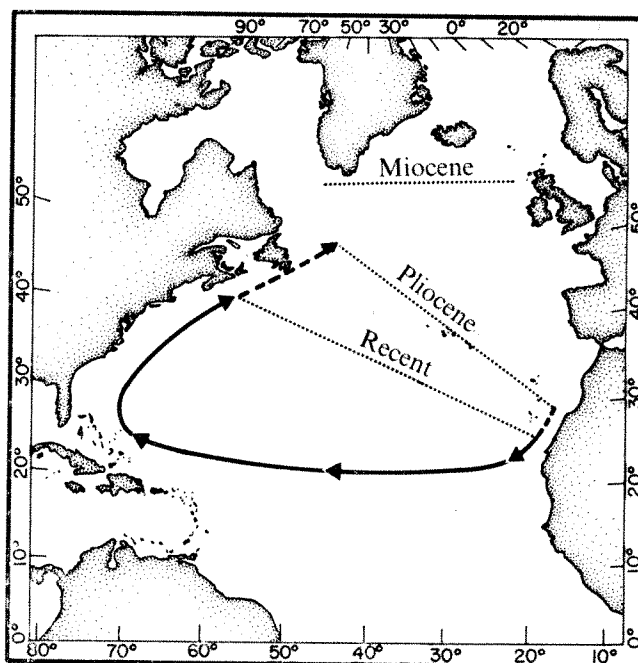


Fig. 1 Clockwise displacement of tropical species in Pliocene and Recent. During the Miocene the northern limits of tropical forms extended much further north on the eastern side of the North Atlantic and E-W distribution may have been symmetrical.

At the end of the Miocene, the Mediterranean was completely closed and there was a 'salinity crisis'. Evaporation was widespread and thick deposits of salt accumulated over the abyssal plain of the entire basin¹³. Although this evaporation must have had a disruptive effect on planktonic faunas, it was of geologically short duration, because the faunal succession across the Mio-Pliocene boundary in the Mediterranean is almost complete. A major biogeographic change is, however, associated with this boundary because the Mediterranean ceased to be part of the tropical regime at the beginning of the Pliocene¹⁴. A lack of tropical forms is evident with the first Pliocene transgression following the 'salinity crisis', but although the possible connection with the salinity crisis is not clear, the coincidence of events seems remarkable.

The disappearance of tropical forms in the Mediterranean was not an isolated regional phenomenon; it was related to a general retreat of tropical forms on the eastern side of the North Atlantic. Tropical forms are also lacking in the early Pliocene sections of the cores drilled in the Hatton-Rockall Basin and the Bay of Biscay^{9,10}. At both those sites early Pliocene

assemblages are composed of a mixture of northern and subtropical forms. Tropical forms do occur in abundance in the core drilled south of the Mediterranean, near the Cape Verde Islands¹⁵. On the western side of the North Atlantic, however, tropical forms still extended much further north during the early Pliocene than they do today. In the core drilled near Labrador, Poore and Berggren¹⁶ recorded *Globorotalia menardii* and other tropical species from sediments no older than early Pliocene. Early Pliocene assemblages consist of a mixture of northern, subtropical and tropical species—a Gulf Stream type of association¹⁰.

These data are sparsely distributed but they clearly fit into a pattern that shows a modern type of dispersal of tropical species, with a strong clockwise northwards displacement on the western side of the ocean (Fig. 1)⁶. There are some intriguing implications, as follows: During the early Pliocene the tropical limit on the eastern side of the North Atlantic retracted to a position south of the Mediterranean entrance, probably close to where it is now. There was no 'cold wave' however, as warm gyral water persisted on the eastern side of the North Atlantic as far north as the Hatton–Rockall Basin (57°N). Early Pliocene assemblages there contain a good representation of subtropical species. Also, the western margin of the Gyre extended perhaps 8° further north than it does today, as indicated by the assemblages recorded in the Labrador core¹⁰.

The early Pliocene therefore, displays an intermediacy between Miocene and modern conditions and most likely represents a critical phase in the evolution of modern ocean climate and circulation. At this time, it seems, the expanded Miocene body of warm gyral water began to contract to modern proportions. What occurred in the early Pliocene was clearly the beginning of a long term condition. Although there have been numerous subsequent climatic fluctuations, there has never been a large enough expansion of warm gyral water to allow the establishment of tropical planktonic foraminifera in the Mediterranean or the higher latitudes of the North Atlantic.

I have omitted any reference to tectonic theory because it is not clear how plate movements would help resolve the questions at hand. Planktonic foraminiferal development displays a curious iterative pattern difficult to account for by unidirectional shifting of plates. For example, it could be argued that the broad expanse of warm water forms seen in the late Cretaceous and Palaeogene was due to an open Tethyan seaway which promoted dispersal from the equatorial region. Yet, a comparable expanse of warm water forms can be seen in the Miocene, when the Tethyan seaway was closed.

It seems that there have been long term cycles in the development of ocean climate and circulation, with alternating expansions and contractions of warm surface water. Expansions are visible in the late Cretaceous, Palaeogene and Miocene and contractions are apparent in the early and middle Cainozoic, which may have involved a complete eradication of warm gyral water. Another contraction seems to have begun in the early Pliocene but in this case there have been no signs of disintegration of warm gyral water, as both tropical and subtropical species have survived several glacial epochs.

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Adaptation to an 8-h shift in living routine by members of a socially isolated community

THE proportion of the working population employed on some form of shift system has increased substantially over the past two or three decades. Expert opinion as to the optimal form of shift system is divided, however, between those who favour 'permanent' systems and those who favour 'rapidly rotating' ones. We have studied the adaptation of half the members of a socially isolated community to a permanent 8-h shift in their routine. Even in this seemingly ideal situation we failed to find complete adaptation of the ~24-h circadian rhythm in either body temperature or performance efficiency over the 10-d study period. This finding casts some doubt on the theory that permanent shift systems are optimal, and that the light–dark cycle is not important in entraining human circadian rhythms.

Circadian rhythms are now known to exist in most physiological parameters¹, and have also been found to affect the efficiency with which man performs various tasks². Performance speed on simple tasks, that place little reliance on short term memory, shows a rise throughout the day (with the exception of a more or less pronounced 'post-lunch decrement'³) and reaches a maximum at about 2000 (ref. 4). This rise parallels that found in body temperature. In contrast, the performance of more complex tasks, which rely heavily on short term memory, reaches a maximum rather earlier in the day⁵, and, with an exceptionally large short term memory load, has been shown to be negatively correlated with body temperature⁶.

In normal circumstances these rhythms are relatively constant from day to day, and are thought to be 'entrained' by a number of environmental *Zeitgeber* (cues) of which the most important seem to be the 24-h light–dark cycle and the 'social' environment⁷. There are, however, two fairly common situations in which man's circadian rhythms are disrupted, namely rapid transition from one time zone to another (the 'jet-lag' effect) and shift work. In the former case, the *Zeitgeber* in the new time zone seem to encourage the adaptation of the traveller's circadian rhythms to the new time zone, and complete adaptation of both temperature and performance rhythms typically occurs within about a

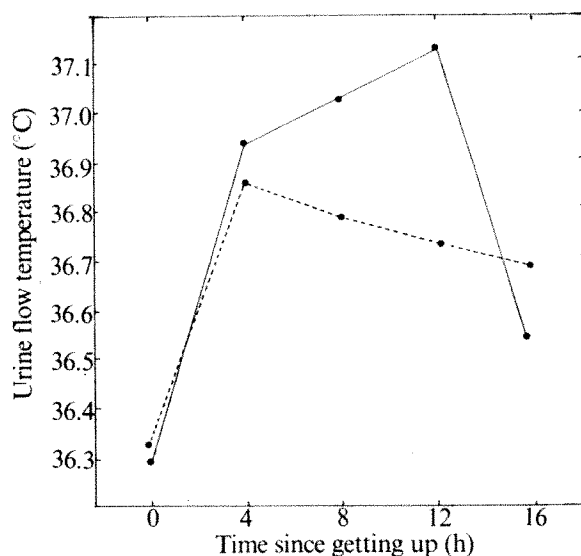


Fig. 1 The 'time since getting up' effect in urine flow temperature for the two pre-shift days (—) and for the 9th and 10th day of the shift period (---). 0, 4, 8, 12 and 16 h since getting up correspond to 0800, 1200, 1600, 2000 and 2400 for the pre-shift days, and to 1600, 2000, 2400, 0400 and 0800 for the shift period days.

week⁷. In the case of shift work, however, and in particular night shift, most *Zeitgeber* remain constant and thus discourage adaptation to the shift worked. Recent work⁸ has indicated that even after 12 successive night shifts (a rather greater number than are typically worked between rest days, during which shift workers normally revert to a 'day-time' routine) neither temperature nor performance is completely adapted to night work. Furthermore, readaptation to the normal routine during rest days seems to be very rapid and to occur within 2 or 3 d.

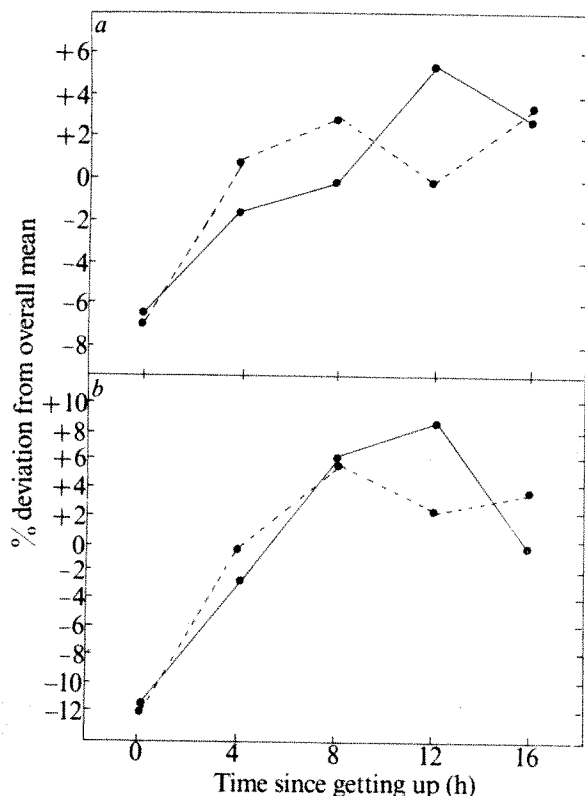


Fig. 2 The 'time since getting up' effect in *a*, manual dexterity and *b*, visual search for the two pre-shift days (—) and for the 9th and 10th day of the shift period (---). The points represent percentage deviation from the overall mean for the day in order to allow comparison between tasks.

'Permanent' night-shift systems, and slowly rotating shift systems that involve a number of successive night duties, thus result in the alternation of slow partial adaptation and rapid readaptation (during rest days) of man's circadian rhythms. In view of this, rapidly rotating shift systems (for example, two mornings, two evenings, two nights and two off) are recommended by some experts. These result in little disruption of the circadian rhythm, but result in the night work being carried out when performance, on at least simple tasks, is at low ebb. Others favour the idea of a permanent 'night subsociety' in which night workers would remain on an inverted routine even on their days off, and should thus be able to adapt completely to the night shift. This view assumes the social *Zeitgeber* to be rather more important than the light-dark cycle, and there is some evidence that this is the case⁷. It would, however, involve a considerable proportion of the population shifting the whole of their normal routine by, for instance, 8 h. This is in contrast to the present habit of most night workers who go to bed immediately after their work, and take their leisure time at the beginning of their waking day.

The present study took advantage of the complete social isolation, but normal light-dark cycle (sunrise ~ 0600, sunset ~ 1530 at the time of the study) that exist during the winter months at South Georgia. Six of the 12 members of

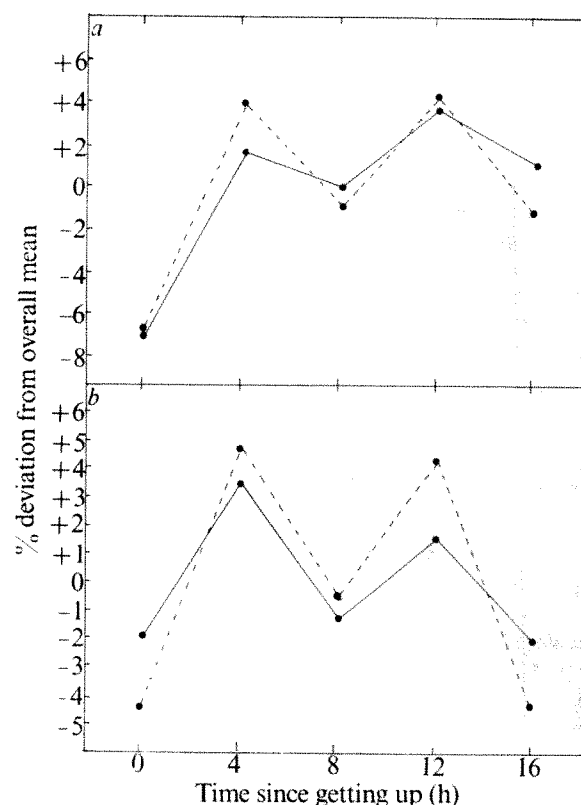


Fig. 3 The 'time since getting up' effect in *a*, arithmetic and *b*, verbal reasoning for the two pre-shift days (—) and for the 9th and 10th day of the shift period (---). The points represent percentage deviation from the overall mean for the day. In spite of considerable practice on these tasks before the study, there was evidence of a practice effect in the pre-shift results. The curves have been adjusted to take account of this using a linear approximation.

a British Antarctica Survey camp at South Georgia (54° 17'S, 36° 30'W) took part in the study. For ten successive days they shifted the whole of their normal daily routine by 8 h, so that instead of sleeping from 2400 to 0800 they slept from 0800 to 1600. For 2 d before, and for the last 2 d of, this 10-d period they undertook four tests of performance efficiency, and measured their urine flow temperatures⁹, at 4-h intervals throughout their waking day. Two of the performance tests used involved a negligible short term memory load: these were a 90-s visual search test involving a search through lines of upright Os for slightly tilted ones, and a manual dexterity test that involved exchanging nuts and bolts attached to two brass plates. The other two tests involved an intermediate memory load and were a 3-min test of verbal reasoning⁵, and a 5-min test of arithmetic².

Since the main concern of the study was whether or not there was complete adaptation (as opposed to some adaptation), the reference point used in the analysis was the time of getting up rather than clock time. If perfect adaptation had occurred there should be no difference in the 'time since getting up' effect from the two stages of the study. If, however, the opposing light-dark cycle, and the social influences of the six members of the camp who did not shift their routine, had prevented complete adaptation then this effect from the two stages should differ.

Considerable adaptation of the temperature rhythm took place, since the two curves in Fig. 1 are not too dissimilar. Indeed, there was an overall 'time since getting up' effect (Anova $P < 0.001$), but there was also evidence that this effect differed for the two stages of the study ($P < 0.025$). Deviation from perfect adaptation at the end of the shift period took the form of a reduction in amplitude, an earlier peak, and the lack of a predictive drop before the sleep

period. As such, it is very similar to that found in earlier shift studies^{7,8}.

The two low memory load tests, manual dexterity and visual search, showed a similar, but somewhat smaller, lack of adaptation (Fig. 2). Although there was no reduction in the amplitude of the 'time since getting up' effect, performance on both tasks reached an earlier peak and failed to show a drop before sleep at the end of the shift period. Again, considerable adaptation had clearly occurred, and only in the case of visual search was there evidence that this adaptation was incomplete ($P < 0.025$).

The two intermediate memory load tasks showed even better adaptation (Fig. 3), and for neither task was there any evidence that the adaptation was incomplete ($P > 0.25$ in both cases). Performance on both tasks reached an early peak 4 h after getting up, and a second peak 8 h later. This double peak has not normally been found⁴, although it has been noted before with an intermediate memory load task⁶. It seems to represent a composite of the early peak shown by pure short term memory tasks, and the late peak shown by simple tasks. The slightly more exaggerated 'time since getting up' effect that these two intermediate load tasks showed at the end of the shift period may be due to partial sleep deprivation. As is typical for night workers¹⁰, some of the present subjects complained of their inability to sleep properly during the shift period, and there is some evidence that sleep deprivation results in more marked daytime effects³. The reason such an exaggeration was not found in the simple tasks may be because such tasks are generally less prone to sleep-deprivation effects¹¹.

The conclusions to be drawn from these results are, in general, very similar to those based on studies where the subjects have not been members of a socially isolated community⁸. Even after ten successive days of an 8-h shift, complete adaptation had not occurred in either temperature or visual search performance. Although the other performance measures showed no significant lack of adaptation, it is generally accepted that different rhythms adapt to a time shift at different rates⁷, and the adaptation of performance rhythms may well be quicker than that of the physiological ones. The lack of complete adaptation could be due to the social influences of the six members of the camp who did not shift their routine, and/or to the opposing light-dark cycle. Although it has recently been suggested that the light-dark cycle is relatively unimportant for man⁷, this is based on results using only an artificial light-dark cycle. It is quite possible that the natural cycle is a rather stronger *Zeitgeber* than these studies would suggest, especially since in the present study the contact between the shifted and unshifted members of the camp was minimal.

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Short term increases in mortality during heatwaves

COMPARISON of the weekly death registrations in Greater London¹ with temperatures at the London Weather Centre during the summer of 1975 shows a temporary increase in mortality that coincides with the heatwave of late July to mid-August (Fig. 1). There was also an increase in death registrations in the first week of June, when there was a change from cold weather with a little snow to very warm weather (but these figures may have been slightly inflated by increased delays in registration following a public holiday in the previous week). Increases in mortality associated with heatwaves have been widely reported in North America, although there is some evidence that the pattern there may have been modified by air conditioning²⁻⁵. Little attention has been drawn to such episodes in England and Wales, although an association between deaths from cerebral infarction and temperature in summer has been reported⁶.

The hot weather during the summer of 1975 was exceptional, yet similar conditions were again recorded in 1976 (Fig. 2). During the heatwave in late June and early July, temperatures were marginally higher than in 1975. Again there was an increase in weekly death registrations in Greater London, predominantly among the over-65 age group. This heatwave affected much of the country; attention has been drawn to a parallel rise in deaths in the remainder of south-east England⁷, and mortality increased for England and Wales as a whole. There was also a small increase in deaths in early May (Fig. 2), when temperatures were high for the time of year. The reduced number of registrations in the week ending September 2 was probably attributable to delays following the public holiday in that week; any similar tendency in 1975 may have been obscured by the further rise in temperature that occurred then.

There are clearly many difficulties in the interpretation of short term changes in mortality based on weekly registration records. This is particularly true in relation to the effects of hot periods, which in England are usually short, although some other major episodes (in 1974 for example), have been associated with marked increases in weekly totals. For a period of hot weather in 1968, however, death totals for Greater London, tabulated by date of occurrence, have been provided by the Office of Population Censuses and Surveys, as part of a general arrangement for a larger study of the effects of environmental factors on mortality (A.M. unpublished). Figure 3 shows the death totals together with daily temperatures measured at London Airport (Heathrow) for this period. Weather data from Heathrow were selected for the main study because of their availability on magnetic tape.

The hot period at the beginning of July was comparatively short lived, and it was associated with warm air currents from the Sahara, which brought an unusually warm night (minimum 20 °C), during which a layer of red dust fell over much of southern and eastern England. This was followed on July 1 by an exceptionally warm day (maximum 32 °C), and another warm night. Similar temperatures were recorded at the London Weather Centre and elsewhere within and beyond Greater London. Mortality increased sharply on July 1 and remained high on the following day. Increases were seen in cardiovascular

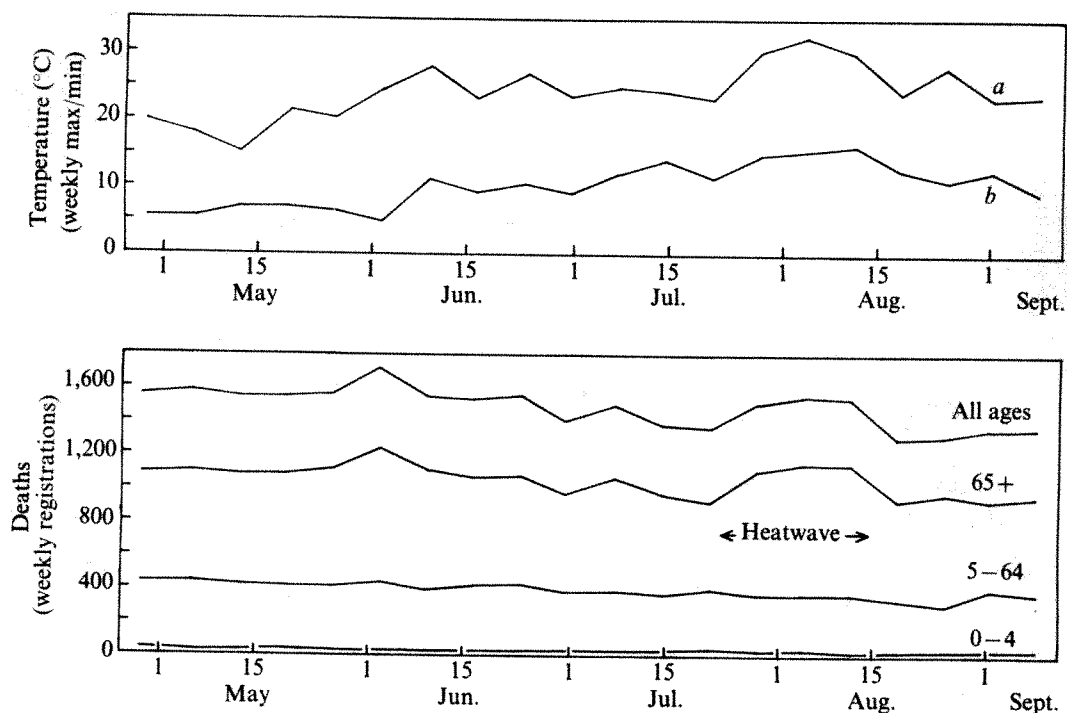
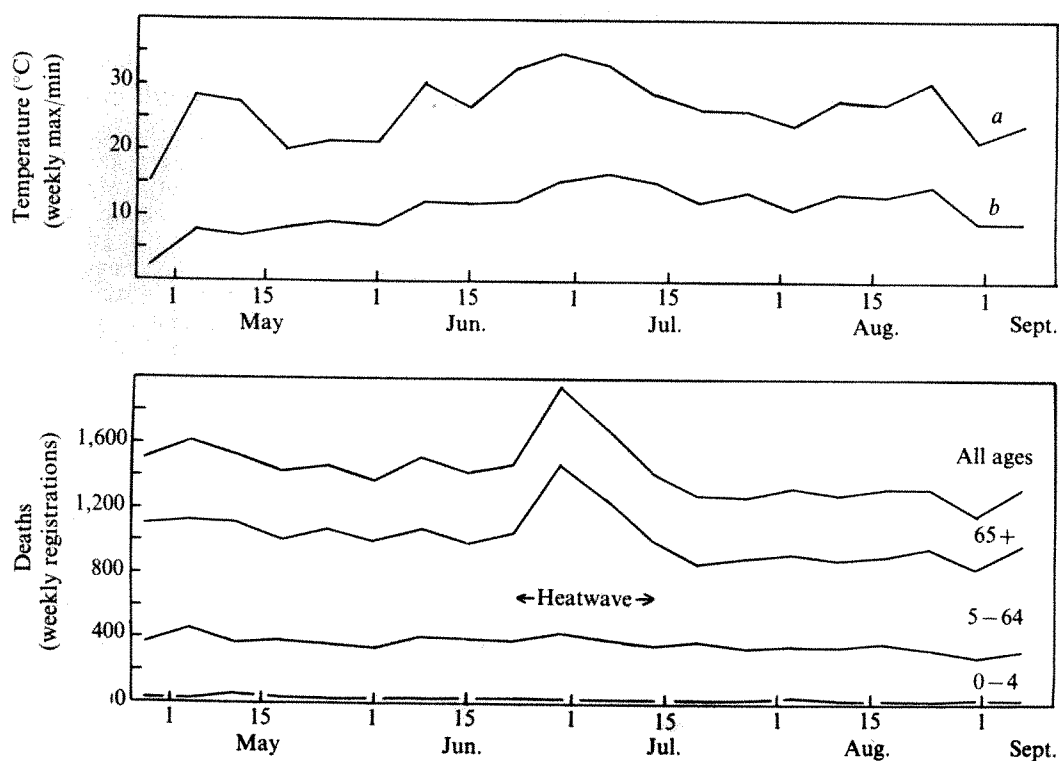


Fig. 1 Weekly totals of deaths registered in Greater London, by age, and weekly maximum (a) and minimum (b) temperatures (London Weather Centre), for summer 1975.

and respiratory causes of death, and in the total of the remaining causes. It is unlikely that the dust associated with this heatwave had any direct effect on mortality. No complete study of the particle size distribution of the dust in suspension was reported, but the mass median diameter

of deposited particles was found to be $9\text{ }\mu\text{m}$ in a study in Hull⁶. This is consistent with unpublished results obtained in London, and these findings indicate that much of the material was too coarse to be inhaled deeply into the lungs.

Fig. 2 Weekly totals of deaths registered in Greater London, by age, and weekly maximum (a) and minimum (b) temperatures (London Weather Centre), for summer 1976.



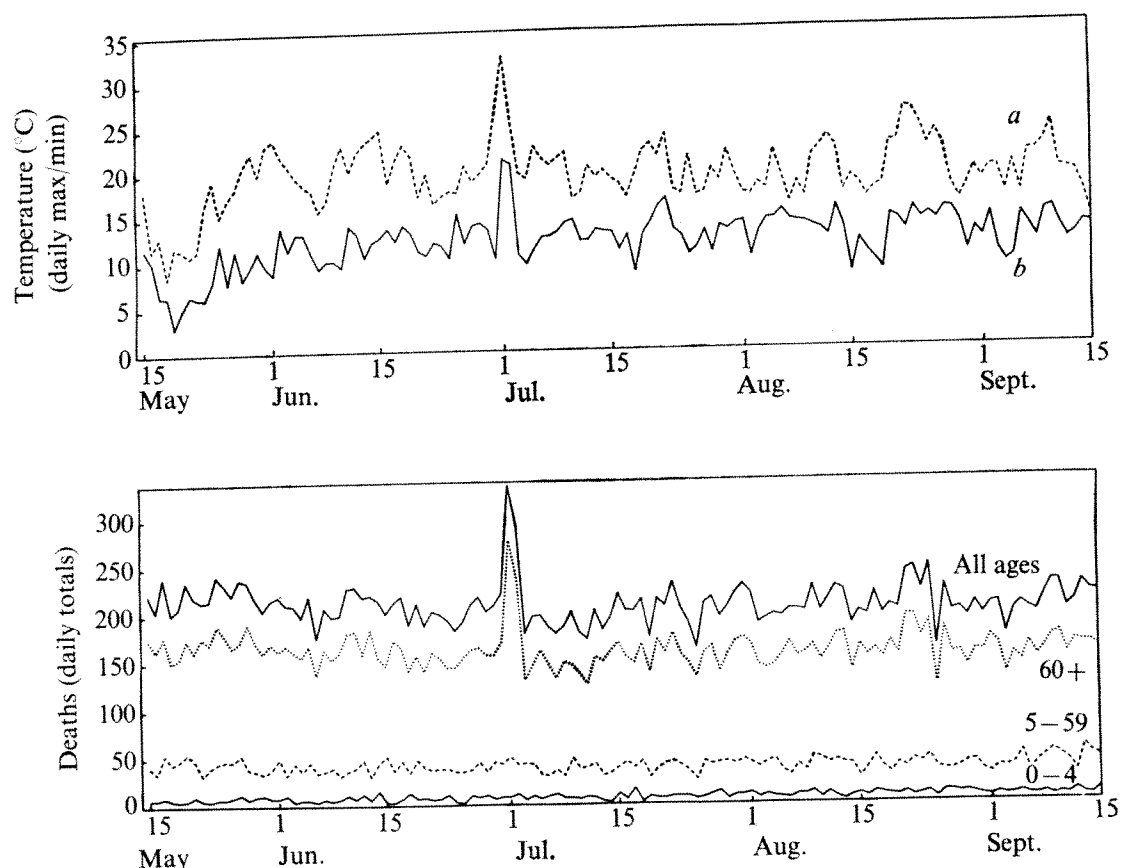


Fig. 3 Daily totals of deaths occurring in Greater London, by age, and daily maximum (a) and minimum (b) temperatures (London Airport, Heathrow) for summer 1968.

An analysis by age at death is included in Fig. 3. Increases in mortality were seen both in the 60–74 and 75+ age groups, but here the two have been combined for clarity of presentation. There was no sign of any increase in mortality in the 0–4 age group (unfortunately it was not possible to subdivide this group retrospectively), in contrast to findings in the United States of increases in infant mortality during heatwaves. The number of deaths per day in this age group in Greater London is, however, very small; it would probably have been necessary to obtain data from a larger population to detect any significant increase in infant mortality.

In other years since 1968, associations have been seen between rises in temperature and increases in daily mortality in the summer months on occasions when the meteorological changes were less dramatic than those described here, and the increases in mortality were smaller; indeed, they were not evident in the weekly death registration totals, and only became evident when the daily totals were examined. This work will be reported in detail elsewhere.

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Mycorrhizal fungi stimulate clover growth in New Zealand hill country soils

VESICULAR-ARBUSCULAR mycorrhizal fungi infect most higher plants and usually increase plant growth and phosphorus uptake, especially in infertile soils¹. Mosse^{2–4} has shown that the indigenous mycorrhizal fungi in some soils are very infective but much less efficient in stimulating phosphorus uptake and plant growth than selected mycorrhizal fungi maintained in glasshouse pot culture. New Zealand soils have a short history of pastoral agriculture (<150 yr) and still range in fertility from very phosphorus-deficient podocarp–broadleaf forest soils to well developed and highly fertile pasture soils. Most hill country pastures consist of introduced grasses and legumes which are invariably mycorrhizal^{5,6}. These pastures have been developed within the past 100 yr from infertile forest soils in which nearly all plants were mycorrhizal⁷ and many were completely dependent on mycorrhizal infection for phosphate uptake and plant growth^{8,9}. Many forests are still being cleared and brought into pasture by oversowing with white clover and spreading heavy dressings of superphosphate. It is important to know whether the indigenous mycorrhizal fungi adapted to these infertile forest soils are as effective at stimulating clover growth in highly fertilised pastures as an efficient mycorrhizal fungus such as *E₃* (ref. 10), a *Glomus* species from Mosse's Rothamsted collection.

Table 1 Hill country soils* in which E_3 was more efficient than the indigenous mycorrhizal fungi

Soils	Soil P† (p.p.m.)	No fungi (controls)	Shoot DM (mg) of white clover plants infected with different mycorrhizal fungi		LSR ($P = 0.05$)
			Indigenous fungi	E_3	
Taihapa silt§	8	30	65	242	1.73
Marua clay†	8	10	75	119	2.74
Marua clay§	12	6	115	118	1.63
Waingaro steep land†	12	11	249	348	1.94
Te Kuiti silt§	12	10	134	315	1.74
Te Pari silt†	12	8	308	413	1.62
Te Pari silt§	24	110	511	944	1.56
Te Pari silt§	32	61	353	734	1.51
Autea clay†	12	17	27	235	1.65
Autea clay§	24	15	117	257	1.92
Autea clay§	24	22	402	557	2.71
New Plymouth brown loam§	16	20	98	235	2.13
Patua loam†	16	24	305	547	2.06
Patua loam¶	28	18	665	673	2.86
Burrell sandy loam†	16	41	36	702	1.61
Mahoenui silt†	16	77	199	367	1.54
Mahoenui silt§	88	607	657	854	1.48
Gisborne sandy loam†	16	25	122	297	3.29
Gisborne sandy loam¶	60	312	476	737	1.70
Mamaku sand¶	20	6	61	97	1.52
Waitakere hill§	24	32	468	634	1.62
Marua hill†	32	329	500	666	1.93
Waikare clay†	48	193	389	393	1.49
Dunmore silt§	120	569	604	682	1.57

*All soils sterilised.

†Measured by modified Truog test¹².

‡Soil from broadleaf forest.

¶Soil from recent or rough pasture.

§Soil from well developed pasture.

Data analysed by analysis of variance after log transformation. When the ratio of any two shoot DM means \geq LSR (least significant ratio), the means are significantly different at $P = 0.05$.

Accordingly, samples of 37 hill country soils were collected in the North Island of New Zealand, in several instances from adjacent forest, recent pasture and well-developed pasture sites on the same soil type. Soils were sieved and most of the sample sterilised with methyl bromide to kill the indigenous mycorrhizal fungi. Most soils had a pH between 5.2 and 5.8. White clover was grown from seed in the unsterilised portion of each soil and at intervals seedlings were harvested and their roots cleared and stained to assess the speed of infection of the indigenous mycorrhizal fungi.

In three infertile forest soils (Waingaro steep land, Waitakere hill, Patua loam) clover roots were only 12, 2 and 1% mycorrhizal 15 d after seed germination, but infection levels gradually rose to 40% after 43 d. In two other forest soils (Autea clay, Burrell sandy loam) the indigenous fungi also infected very slowly with only 6–11% of the roots mycorrhizal even after 43 d. In all the remaining soils, including the Waitakere, Patua, Autea and Burrell soils from under well developed pasture, the indigenous mycorrhizal fungi in the pasture were very infective, with 30–90% of clover roots mycorrhizal within 15 d of seed germination. It is apparent therefore, that during the change from forest to pasture, soils not only become more fertile (Table 1) but their mycorrhizal fungi infect clover more rapidly.

In each of the 37 soils, clover was grown from seed for one month in the unsterilised portion, and seedlings became infected with the indigenous mycorrhizal fungi. Seedlings were then transplanted two per pot into 10-cm pots of the same soil which had been sterilised.

For comparison, seedlings which had been raised in E_3 -infested sterilised soil and control seedlings in sterilised soil with no added fungi were also transplanted into pots of each sterilised soil. Twice filtered washings from all unsterilised soils were added to the pots to ensure that all plants received the same microflora apart from mycorrhizal fungi.

All plants were inoculated twice with *Rhizobium trifolii* strain TA₁ and received weekly applications of nutrient solution containing all essential elements except N and P. This ensured that P alone was limiting growth. There were four replicate pots of each of the 111 treatments (37 soils \times 3 mycorrhizal regimes) randomly arranged and the experiment ran for eight weeks in a shaded glasshouse.

Tables 1 and 2 show that most of the indigenous mycorrhizal fungi were quite efficient and greatly increased growth over controls, especially in the infertile forest soils in which responses were up to 38-fold (Te Pari silt loam, 12 p.p.m. P, Table 1). In all but four soils, 30% or more of the root system was mycorrhizal.

In Burrell sandy loam (forest, 16 p.p.m. P) however, the indigenous fungi had only infected 8% of clover roots and mycorrhizal plants were smaller than controls. In Autea clay (forest, 12 p.p.m. P), the indigenous fungi had managed to infect 39% of clover roots, but were very inefficient and hardly increased shoot DM over controls. In both these soils, inoculation with E_3 led to massive increases in shoot dry matter (DM) (Table 1). In the other three forest soils in which the indigenous mycorrhizal fungi had been initially slow to infect, root infections were high and growth responses to indigenous mycorrhizal fungi were large.

Although the response to mycorrhizal infection decreased as soil fertility rose (Tables 1 and 2) the indigenous mycorrhizal fungi were still beneficial in most of the fertile soils, with a growth response of 39% occurring in Burrell sandy loam from pasture (120 p.p.m. P, Table 2). In five soils, the growth responses to the indigenous mycorrhizal fungi were not significant at the 5% level although they followed the general trend.

In 24 soils, including most forest soils, E_3 was more efficient than the indigenous mycorrhizal fungi at increasing plant growth (Table 1). E_3 infection caused 1,940% more plant growth than the indigenous mycorrhizal fungi in Burrell sandy loam from under forest (16 p.p.m. P, Table

Table 2 Hill country soils* in which the indigenous mycorrhizal fungi were more efficient than E_3

Soils	Soil P (p.p.m.)	Shoot DM (mg) of white clover plants infected with different mycorrhizal fungi			LSR ($P = 0.05$)
		No fungi (controls)	Indigenous fungi	E_3	
Te Kuiti silt§	12	10	355	156	1.59
Whangamomona hill§	12	12	269	151	1.75
Waitakere hill‡	16	62	432	408	1.71
Marua hill§	20	90	459	369	1.99
Aria silt§	24	52	866	707	1.97
Patua loam§	44	37	783	352	2.19
Oruanui hill§	44	137	852	837	1.88
Marua hill¶	56	33	528	498	2.79
Mahoenui silt§	48	447	1,013	667	1.42
Oropi sandy loam§	64	759	1,268	997	1.42
Waikare clay§	108	597	727	631	1.28
Gisborne sandy loam§	108	1,060	1,230	1,169	1.31
Burrell sandy loam§	120	988	1,373	1,139	1.39

*All soils sterilised.

‡, ¶, § as in Table 1.

1), and in excess of 100% more growth than the indigenous fungi in seven other soils, and 30% more growth in a total of 20 soils (Table 1).

The efficiency of a mycorrhizal fungus at increasing plant growth was not related to the percentage of host roots it infected. For example, in Taihape silt loam the E_3 -infected plants had 55% of roots mycorrhizal and a mean shoot DM of 242 mg, while plants infected with the indigenous mycorrhizal fungi had 73% of mycorrhizal roots but only 65 mg shoot DM. For most soil types, the increased efficiency of E_3 over the indigenous mycorrhizal fungi was greatest in the forest soil, but in Te Pari silt loam, the reverse was true.

In 11 soils the indigenous fungi were more efficient than E_3 (Table 2), but in only four soils (Te Kuiti, Whangamomona, Patua and Mahoenui) was the increased growth greater than 30%.

In conclusion, it is apparent that clover is highly dependent on infection by mycorrhizal fungi for growth in many hill country soils. The extra benefit from E_3 over the indigenous mycorrhizal fungi achieved in such a wide range of soils is very promising, and field inoculation may be feasible if E_3 can be established in natural soils in the face of competition from the indigenous but less efficient fungi already present. Mosse⁴ has already demonstrated this in some British soils.

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Protozoa as sources of antigen in 'humidifier fever'

IN the field of hypersensitivity reactions to inhaled materials, sources of antigenic production are as varied as avian protein, pituitary stuff, wheat weevil, *Aspergilli*, and thermophilic actinomycetes¹. We have found a completely new group of organisms

which give rise to antigens associated with 'humidifier fever'²—one of the diseases in the extrinsic allergic alveolitis group¹. The organisms are amoebae that develop in the recirculating water used in the humidification systems.

The ubiquitous nature of these Protozoa suggests that sensitisation to them or their products may not be confined to the groups of individuals already studied, and that industry should be made aware of the possibility of the development of these organisms in stagnant or recirculating water, particularly when processes release material upon which microorganisms can develop into the environment.

Our attention was first drawn to the problem when about 20 of 50 office staff were reported as having pyrexial episodes, often with myalgia, some hours after leaving the factory, and usually on a Monday. The office adjoins the factory which uses a humidifier for plant heating as well as for one of its industrial processes. Moreover, above the office is a suspended ceiling on which dust from the factory has accumulated.

Such episodes have been recorded previously as 'humidifier fever'² or as hypersensitivity pneumonitis attributable to air conditioner contamination,³ although the clinical manifestations are not necessarily identical with those we have found.

It is considered that contamination of the humidifiers by organisms, and the release of these or their products into the environment is responsible for sensitisation and subsequent disease⁴. The source of antigens has been described as a member of the *Micropolyspora* genus³ (now identified as *Thermoactinomyces vulgaris*—J. Salvaggio, personal communication), *T. vulgaris*⁴ or thermotolerant bacilli⁵. Lines of precipitation have been recorded against *T. vulgaris* with sera from affected individuals⁴, and inhalation challenge tests using *T. vulgaris* have produced a pyrexial episode^{3,4}. However, some *T. vulgaris* strains can produce materials capable of reacting nonspecifically with human sera⁶, as can material from *Staphylococcus aureus*⁷ and the so-called byssinosis 'antigen'⁸. Apart from this possibly 'false positive' reaction, serological testing with extracts of other organisms has been without success^{9,10}, although lyophilised water from the humidifier may contain materials capable of reacting with sera of affected individuals¹⁰.

Our studies involved isolating organisms from the atmosphere of the office, the dust on the false ceiling, and from the humidifier. Many fungi and bacteria were isolated, identified, and extracts were tested against sera from affected individuals. Apart from one or two weakly reacting extracts, results were negative on gel diffusion, although extracts of the 'ceiling dust' and material from the humidifier baffle plates reacted strongly with the sera of 16 of 18 affected individuals and 2 of 18 unaffected individuals working in the office ($P < 0.001$).

When material from the baffle plates was viewed microscopically it became apparent that Protozoa were present in abundance. Isolation provided two main groups of

Protozoa—ciliates and amoebae. Purified ciliates failed to produce antigenic material that reacted with sera from affected cases, whereas amoebae produced up to five precipitin lines. A named amoeba, *Naegleria gruberi* was obtained (Culture Centre of Algae and Protozoa, Cambridge), and grown in monoxenic culture with *Klebsiella aerogenes*. The resulting extract was antigenic, and the gel diffusion reaction indicated antigenic identity with material in the ceiling dust (Fig. 1). Amoebae isolated from the ceiling dust produced a similar result; *K. aerogenes* extract was weakly positive.

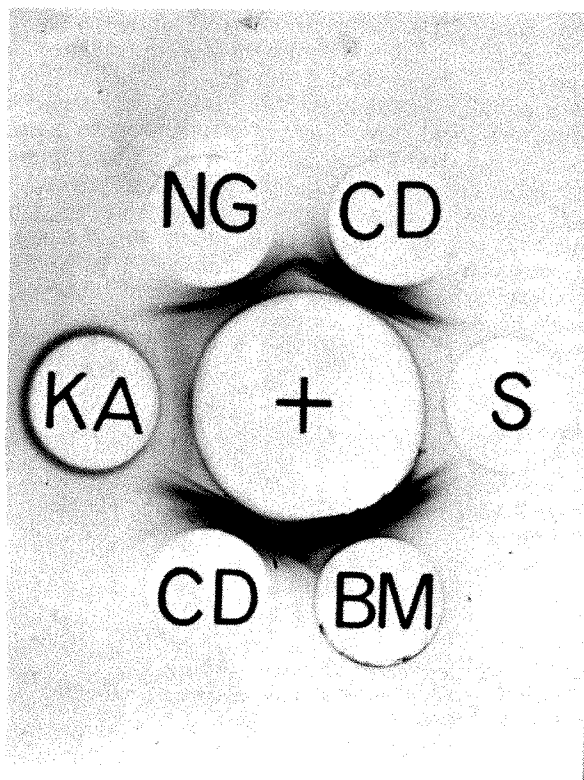


Fig. 1 Gel diffusion reaction: +, pooled sera from affected cases; S, saline; CD, ceiling dust extract; BM, Baffle plate 'muck' extract; KA, *Klebsiella aerogenes* extract; NG, *Naegleria gruberi* extract; CD and BM were aqueous extracts of ceiling dust and baffle plate muck concentrated and dialysed.

N. gruberi was grown for 7 d at room temperature on non-nutrient agar streaked with live *K. aerogenes*¹¹ and the plates extracted with 1% aqueous phenol, then concentrated and dialysed. *K. aerogenes* was prepared by the same method as *N. gruberi*. Note the join of the line from *N. gruberi* and CD indicating antigenic identity.

Thus, we believe we have been able to determine a source of antigens associated with 'humidifier fever' that has hitherto remained unrecognised; possibly one species of amoeba, that is, *N. gruberi*, will not provide all the antigens to which affected cases respond serologically; indeed, our current work suggests that several amoebae are present. We hope to assess the individual contribution of each of these to the overall antigenic picture.

We are indebted to Drs A. Jones and G. Westhall of the E.M.A.S. for first bringing the cases to our attention, and to Dr A. Axford for his help in clinical epidemiology. The photographic expertise of D. Llewellyn is appreciated.

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Suppression of murine lupus erythematosus by Dactinomycin

THE disease of the NZB×NZW/F₁ hybrid mouse (B/W) is a model of systemic lupus erythematosus (SLE), perhaps the most autoimmune of human diseases. The animals develop LE cells and anti-nuclear antibodies of various specificities, and die of a fulminating chronic glomerulonephritis at an early age¹. Abnormalities of the skin² and central nervous system³, also reminiscent of clinical SLE, are present as well. Like SLE patients, many of the mice have greatly extended lifespans under treatment with corticosteroids, azathioprine or cyclophosphamide, or combinations thereof^{4,5}. There is a body of evidence⁶ which suggests that aberrant availability of autoantigens may be a factor in both SLE and B/W disease. Other groups have emphasised the availability of nuclear materials from keratinising skin cells as a source^{6–8}, but it seems to us that the nuclei from maturing erythrocytes should also be considered. All of the immunosuppressive agents mentioned above suppress erythropoiesis in mice⁹ and may also be limiting access of

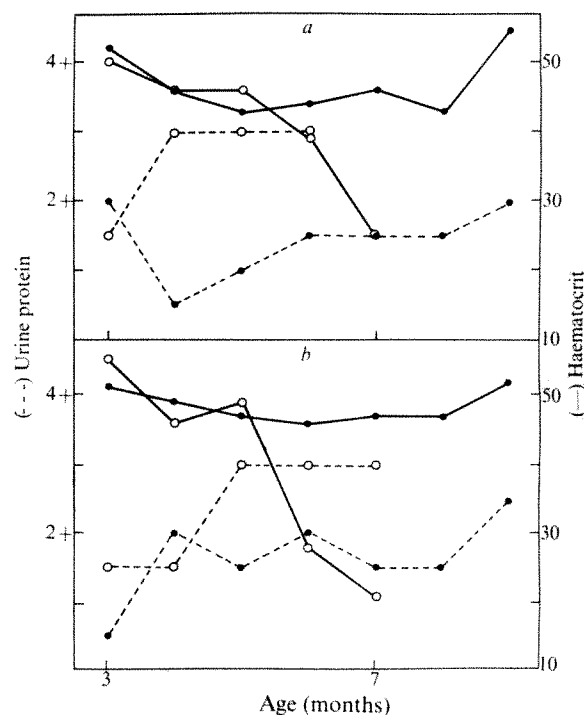


Fig. 1 Longevity of Dactinomycin-treated NZB/NZW mice to 405 d. Control deaths began at about 6 months of age, and the last control died at close to 12 months. The cut-off on the graph was arbitrary, but all treated mice were alive beyond 450 d.

antigen to immune complexes. Here we show that Dactinomycin (actinomycin D), which inhibits erythropoiesis at doses which do not suppress antibody formation (ref. 9 and unpublished observations), is nevertheless an effective suppressant of B/W mouse disease.

In the present series female B/W mice were treated with Dactinomycin, beginning with a small daily dose, 0.35 μg subcutaneously, at about 3.5 months of age, increased to 1.8 $\mu\text{g d}^{-1}$ about 6 weeks later and doubled to 3.5 $\mu\text{g d}^{-1}$ a month after that, at about 6 months of age. The adjustments reflected significant reticulocyte counts in most of the treated animals. Weight was checked twice weekly; and

haematocrit, reticulocyte count, and urine protein every 4–5 weeks. The drug was stopped when weight loss was consistent in the group; treatment was suspended for an average of 5–6 d a month. Post-mortem tissues were fixed and snap-frozen for light and fluorescence microscopy.

At the present time, all 11 Dactinomycin-treated animals are alive at 14 months of age or more, mean 455 d (range 444–460), while all 11 age-matched control animals have died at a mean age of 256 d (range 189–358 d). Figure 1 illustrates the longevity data to 405 d. The difference is significant by the sign test ($P < 0.001$)¹⁰ and by the Mann-Whitney test ($P < 0.01$)¹¹. The treated animals have generally had low (or no) reticulocyte counts, but have maintained haematocrits at 40 or above. Urine protein at 14 months of age was 2+ and 3+ in single animals, but less in the other nine.

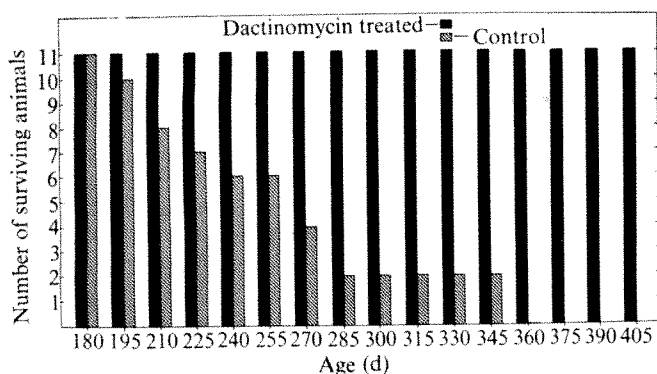


Fig. 2 Urine protein (-----) and haematocrit (——) in matched Dactinomycin-treated (●) and control (○) mice. The control animals died *a*, at 215 and *b*, at 230 d of age. The inverse relationship of haematocrit and urine protein is commonly seen in untreated animals. Haematocrits of treated animals have rarely been below 40, or urine protein above 2+. These trends continue.

Figure 2 illustrates the course of two age-matched pairs of animals, treated and untreated, from 3 to 9–10 months of age. Both show the stability of haematocrit and urine protein levels in the Dactinomycin-treated mice, and the inverse relationship of urine protein and haematocrit in the control animals. All untreated animals had heavy proteinuria before death and eight out of 11 had anaemia. Post-mortem renal tissue was evaluated by two observers without knowledge of source, and was judged 3+ or 4+ in severity in all the control animals. Granular staining of immunoglobulin and complement was seen in the glomeruli, with abundant mesangial deposition in most instances and varying amounts of glomerular loop staining. Both the light and fluorescence microscopic findings were typical of our experience in other series.

It seems from this initial group that Dactinomycin alone may suppress B/W disease as well as cyclophosphamide does and probably better than corticosteroids or azathioprine or both. Though a little unkempt in appearance because of loss of hair at injection sites, the animals are in a good condition, with mild or less proteinuria, at well over a year of age, and we plan to continue treating them for their remaining lifetimes.

The majority view of B/W disease today probably includes the following elements: inability to develop or sustain immunological tolerance¹; diminished cell-mediated immunity, following early loss of suppressor cells^{1,4}; and activity of both the Gross and FMR (Friend–Moloney–Rauscher) oncornavirus group^{1,4,12,13}. There has been little evidence that Dactinomycin facilitates tolerance induction¹⁴; in fact, Claman *et al.*¹⁵ found that Dactinomycin administration to mice converted soluble bovine gamma globulin from a tolerogen to an immunogen. In the few studies of cell-mediated immunity in Dactinomycin-treated

animals, responses were neither suppressed nor enhanced^{16,17}. Dactinomycin does have profound effects on the early phase of both Friend and Rauscher leukaemia^{18,19} at the same doses which inhibit normal erythropoiesis, presumably reflecting diminished numbers of erythroid target cells rather than direct effects on the viruses²⁰.

Another possibility is that combination of Dactinomycin with DNA inhibits reactivity with antibody to DNA. There are data suggesting that this is unlikely: Carr *et al.*²¹ developed an assay for human antibody to DNA which employed labelled Dactinomycin–DNA as antigen. They explicitly ruled out any influence by the intercalated Dactinomycin on reaction with antibody.

Although our data indicate that we have suppressed both erythropoiesis and B/W disease by Dactinomycin administration, other mechanisms cannot be ruled out. Immunosuppression, immunopotentiality, anti-viral effects, and interference with antigen–antibody combination have been mentioned, and there may well be others. On the basis of what was known, the profound influence on B/W disease was unexpected, and it suggests that re-examination of the therapeutic potential of Dactinomycin is warranted.

Our mouse colony originated from stocks held at the University of Minnesota, Minneapolis, Minnesota. Merck, Sharp and Dohme donated Dactinomycin. We appreciate the assistance of Miss June Smith, Department of Laboratory Medicine and Pathology, in setting up our facility and training our personnel. We thank Mr Ulrich H. Rudofsky and Dr Rodrigo E. Urizar for reviewing the histology, and Mr Andrew D. Simmons for his assistance with the immunofluorescence microscopy.

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Hyperplastic nodules after portacaval anastomosis in rats

FOCAL proliferative or hyperplastic regions of liver cells are readily recognised in human liver diseases^{1,2} and there is a possibility that some types of hyperplastic nodules are important for the development of liver cancer³. The case for the precancerous nature of hyperplastic nodules however, rests entirely on data derived from chemical hepatocarcinogenesis and it is striking that toxic chemicals which are

not known to lead to cancer do not induce hyperplastic nodules, whereas almost all hepatic carcinogens do give rise to nodules¹. It seems of interest therefore to report the regular development of hyperplastic nodules in rats subjected to portacaval anastomosis without the addition of a known carcinogen to the diet or drinking water.

Male white rats bred in the Sutton Bonington Agricultural Unit, University of Nottingham, were maintained on Diet 41B supplied by Lillico Ltd. They were subjected to portacaval anastomosis (PCA) or sham operation² under ether anaesthesia at 150–200 g and housed six to a cage, on wire grids after the second postoperative day. PCA animals in groups of seven and controls in groups of three were killed after 1 week, 6 weeks, 2, 4, 6, 9 or 12 months. A further six rats were killed at 18 months and six were subjected to partial hepatectomy at 12 months and killed 5–8 months later. Apart from two PCA and one control rat in the first seven groups which were kept for glutaraldehyde perfusion, the liver of each rat was suspended in formol-saline and thin slices were fixed also in formol-alcohol. Dye was injected into the spleens of two controls, two 4-month PCA and three 6-month PCA to check for the presence of a collateral portal flow into the liver³.

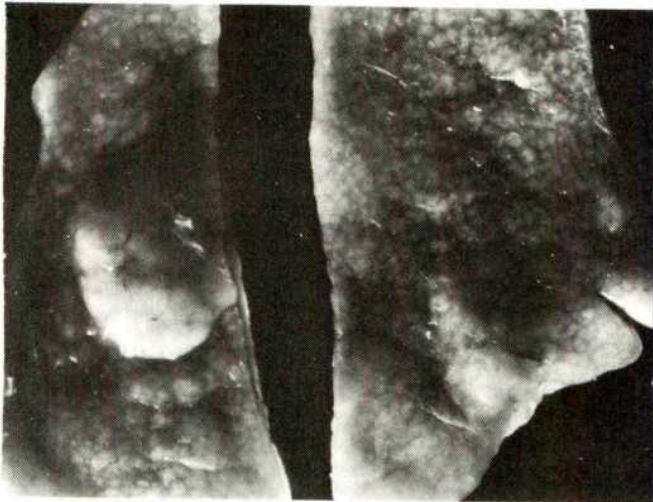


Fig. 1 Hyperplastic tissue extending beyond inferior surfaces of liver 4 months after PCA. Pattern elsewhere regular ($\times 4$).

The animals recovered quickly from the operation and body weight was reduced for about a week, after which the weight gain paralleled that of controls. Liver weights were consistently lower (mean approximately 2% of body weight compared with approximately 3.5% in controls). The anatomical markings were more distinct after 1 week and by 6 weeks the liver surfaces were mottled dark and pale pink. These contrasting markings were accentuated by the second month and although many pale regions were regular but convex by 4 months, confluent foci were found and measured up to 1.0 cm in diameter (Fig. 1). By 6 months such hyperplastic foci varied in size and were found in all PCA animals; in two out of seven animals, masses extended beyond the borders of the median or anterior lobes of the liver. By 12 months, in every instance, the borders and surfaces of the livers were distorted by pale irregular hyperplastic regions, and masses, sometimes polypoid, projected into the peritoneal cavity (Fig. 2). Markedly irregular nodularity was striking after 18 months (Fig. 3).

Microscopy showed that the foci at first consisted of parenchymal cells which contained normally arranged vessels separated by atrophied cells in the perivenous regions, with evidence of compression by 6 months and loss of normal vascular relationships at 12 and 18 months (Fig. 4). Foci of crowded cells were noted, but no unequivocal malignant transformation was detected. Early

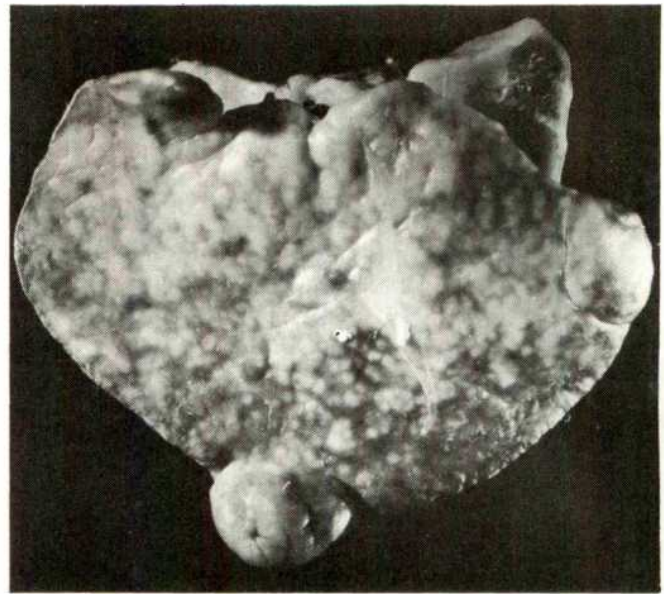


Fig. 2 Much distortion of liver surface by hyperplastic tissue, with nodule on inferior border of median lobe. Pattern elsewhere irregular. 12 months after PCA ($\times 2.3$).

changes were found in all experimental animals but not in controls and later changes with obvious distortion were noted to a lesser or greater extent in all PCA animals. Although adhesions were found quite frequently, portal revascularisation was not demonstrated on dye injection. The changes were more marked in animals subjected to partial hepatectomy in addition to portacaval anastomosis.

These experiments form part of an investigation into the reactions of atrophied hepatocytes. Such atrophied cells are known to undergo hyperplasia after the sudden reduction of hepatic tissue effected by partial resection in the PCA rat⁷. It therefore seemed possible that the rather slower, but nonetheless substantial, loss of parenchyma inherent in the response to the uncomplicated PCA also might effect a stimulus to hyperplasia. Such hyperplasia as occurred in this series seemed to involve at first predominantly periportal cells and it is not clear what part such anatomical



Fig. 3 Large nodule formation in liver remnant 18 months after PCA and 6 months after partial hepatectomy ($\times 2.3$).

zation might play in the ultimate development of hyperplastic nodules. The appearance of the hyperplastic nodules is similar to those induced by chemical carcinogens, but it is not yet known whether similar changes in biochemical properties and the antigenic complement of the hepatocytes occur^{3,4}. It would clearly be of major interest to ascertain whether a preneoplastic antigen is detectable in these circumstances³.

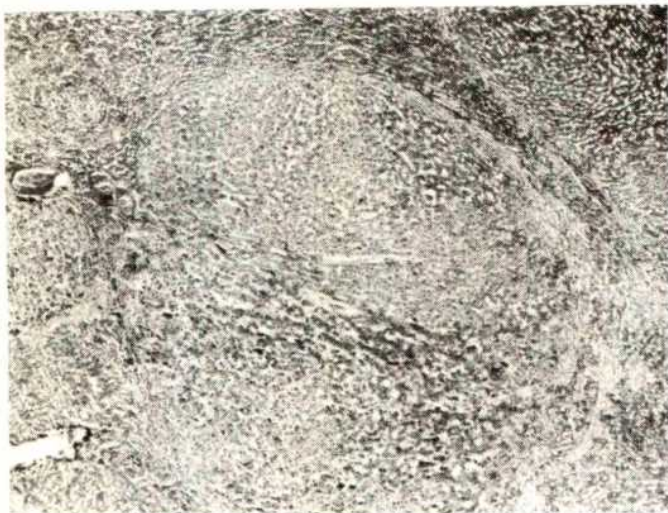


Fig. 4 Irregular hyperplastic nodule with compression of adjacent parenchyma and distortion of hepatic plates, taken from liver shown in Fig. 3. Haematoxylin and eosin ($\times 33.6$).

It is puzzling that these changes have not been mentioned previously^{6,8-10}. Most papers report that the microscopic appearances of livers from PCA rats are remarkably normal and that it is difficult to distinguish these from control livers. Microscopy of the present series confirms this superficial impression in early cases and, for the most part, vascular relationships are retained and plates of hepatocytes are flanked by sinusoids with a good complement of littoral cells^{6,9}. It is only when a comparison is made with macroscopic appearances that the irregularity, which is much more striking macroscopically and sometimes difficult to define microscopically particularly before 6 months, can be identified. After longer periods the compression of these foci on adjacent parenchyma is clearer and eventually the vascular pattern is much distorted, but this is a late phenomenon. At this stage, microscopy is revealing but only if the relevant part is selected. It seems possible therefore, that the changes described may easily not be noted unless gross examination is combined with microscopy.

Other possibilities include strain differences in the rats, housing and dietary effects. Strain differences are difficult to evaluate but in common with most other colonies none of our controls showed evidence of a tendency to nodule formation and at least one report refers to a similar strain⁶. Animal housing is relevant to the well-being of the experimental animals¹¹ and wire-bottomed cages much improve the clinical state of the rats, but there is no suggestion in this or any other work that this form of caging is conducive to nodule formation. It is difficult to see how the reduction in coprophagy might effect an increased rate of DNA synthesis, by pathways involving a reduced B₁₂ metabolism or any others. With regard to the possibility of dietary effects, it is clear that PCA animals in general have a lower dietary intake and the animals in this series were no exception. The rate of increase in body weight was, however, comparable with that of controls and similar to that described for all other series. Pair feeding was not an effective procedure in a long term experiment involving wire-bottomed cages, because substantial quantities of food were noted in the waste and accurate quantitative recovery was

not carried out. There were no conventional stigmata of dietary deficiency on clinical or microscopical examination and the incidence of intercurrent disease was at the same rate in experimental and control rats, occurring for the most part in old animals.

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Induction of oncogenic transformation *in vitro* by ultraviolet light

SKIN fibroblasts derived from patients with the autosomal recessive disease *xeroderma pigmentosum* are defective in excision repair of photodamage induced by short wavelength ultraviolet light¹. In conjunction with the clinical observation that these patients are extremely prone to develop skin cancer², it has been suggested that DNA repair plays a central role in the process of carcinogenesis. Although methodologies are available for investigating the molecular biology of mammalian DNA repair, studies of carcinogenesis induced by UV have been limited largely to the use of the hairless mouse³⁻⁵. In a preliminary report, DiPaolo and Donovan⁶ reported the induction of transformation in primary hamster embryo cell cultures by ultraviolet light. Mondal and Heidelberger⁷ reported inducing malignant transformation in mouse 10T1/2 cells *in vitro* by exposure to ultraviolet light followed by treatment with tetradecanoyl phorbol acetate. We report here that ultraviolet light of 254-nm wavelength alone is sufficient to induce reproducible transformation in this cell line, and we present the dose-response relationship for this phenomenon.

We used the C3H mouse-embryo-derived cell line designated 10T1/2 clone 8 (ref. 8). Stock cultures were carried in large glass bottles in Eagle's basal medium supplemented with 10% heat-inactivated foetal calf serum (Gibco) and antibiotics. Cells were in passages 11-15. Transformation was scored as previously described^{9,10}. Only type III foci were scored as transformants: these foci grow in a multi-layer, criss-crossed fashion over a background monolayer of normal cells, and exhibit a highly polar, fibroblastic morphology. When injected into syngeneic mice, they cause fibrosarcomas^{10,11} while normal cells do not. The transformed foci we have obtained with ultraviolet light do not differ in morphology from those obtained with X-ray⁹ or

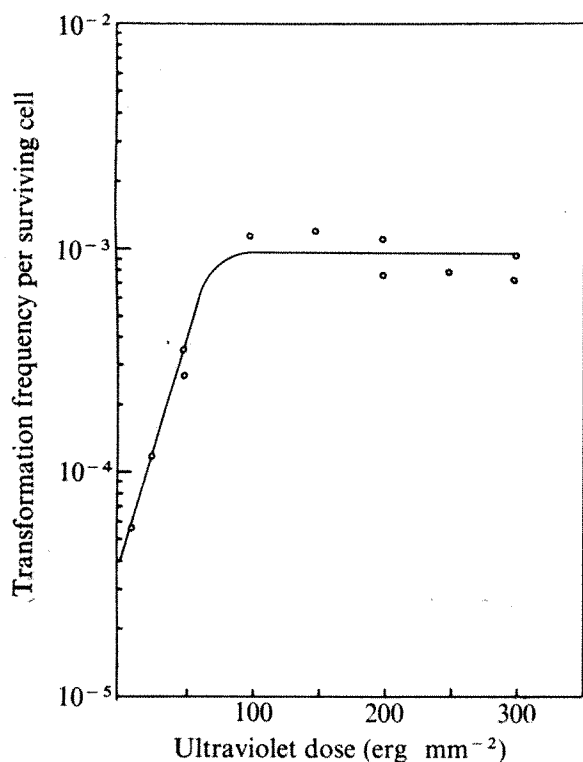


Fig. 1 Transformation frequency per surviving cell as a function of UVL dose for the 10T1/2 mouse fibroblast cell line. 100-mm plastic dishes (Falcon) were seeded with 300–400 surviving cells each to allow them to develop approximately 14 doublings before confluence was reached⁹. The medium was removed 20 h after plating and the cells were irradiated by a bank of five GE G8T5 tubes emitting predominantly 254 nm ultraviolet light at a dose rate of $4.5 \text{ erg mm}^{-2} \text{ s}^{-1}$ at the cell surface. After irradiation, cells were overlaid with fresh medium and returned to a 5% CO_2 humidified incubator held at 37°C . The medium was then changed twice weekly until confluence, then weekly until the seventh week, at which time the cells were fixed with Bouin's solution, stained with Trypan blue and scored for transformants. For each data point, 20–60 transformation plates were used while four plates, each seeded with a cell density one-fifth that of the transformation plates, were terminated at the end of the 2nd week to assay for the number of cells surviving each treatment.

chemical carcinogens^{10,12}. No type III transformants were seen in unirradiated control cultures in any of these experiments.

Figure 1 presents the dose-response curve for ultraviolet-induced transformation. The transformation frequency on the ordinate is plotted as transformants per surviving cell. Clearly this transformation frequency rises initially at low doses, but reaches a plateau after an inflection point at about 75 erg mm^{-2} . The existence of a plateau implies that over this higher dose range a constant proportion of the surviving cells (as assayed by the ability to proliferate indefinitely) is transformed. Thus, when the results are expressed as transformants per irradiated cell (Fig. 2, lower curve), the portion of the curve which corresponds to the plateau is parallel to that part of the dose-response curve for survival (Fig. 2, upper curve). In accordance with our assertion that both survival and transformation are cell-mediated processes¹¹, these data are consistent with the hypothesis that beyond a limiting amount of damage, the relative efficiencies of the cellular repair mechanisms which mediate survival and transformation remain constant. Transformation is genetically selected neither for nor against.

The plateau region in Fig. 1 spans a dose range occupied by both components of the biphasic survival curve (Fig. 2, upper curve). The second component has been shown to represent a subpopulation consisting predominantly of cells

in the G_2 phase of the cell cycle¹³. Host-cell reactivation studies of ultraviolet-irradiated herpes-virus¹⁴ have suggested that the superior survival characteristics of this subpopulation, as evidenced by a greater than twofold increase in the D_0 of the survival curve, can at least in part be attributed to additional repair processes that are operational only in these cells. Our data suggest that no selective advantage has been conferred on this subpopulation with respect to transformation, raising the possibility that the additional repair responsible for the second component of the survival curve represents increased activity of existing processes rather than the operation of a new process. For example, the operation of a more or less error-prone repair mechanism does not seem to be involved.

At the low dose region, although there is an initial shoulder on the survival curve, the curve for transformation per irradiated cell (Fig. 2, lower curve) has an initial

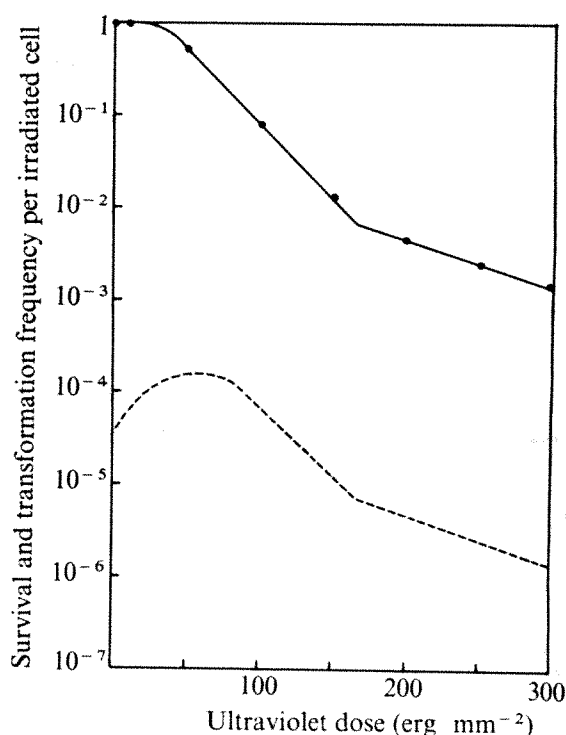


Fig. 2 Dose-response curve for survival (●) and transformation frequency (—) per irradiated cell.

positive slope, at least at doses greater than 10 erg mm^{-2} . It is the combination of the two processes of transformation and cell killing that produces the initial rise and the subsequent decline of this curve. These results are thus qualitatively similar to those for X-ray-induced transformation^{9,15} and other experimental evidence for the existence of a most efficient dose for radiation-induced cancer.

We propose that the transformation seen in these experiments results from the operation of the postreplication repair mechanism which is thought to be error prone. Excision of pyrimidine dimers in the 10T1/2 cell line has been shown to be below the level of detection (personal communication from J. S. Bertram). Moreover, since the cells in our experiments were actively traversing the cell cycle at the time of exposure to ultraviolet light, one can envisage that the presence of unexcised dimers at the first postirradiation DNA synthesis would necessitate the operation of the postreplication repair mechanism. The operation of this mechanism would introduce errors into the genome, some of which would lead ultimately to the fixation of transformation^{16–19}.

Our results suggest that established cell lines such as the 10T1/2 mouse fibroblast might be used to study quantitatively ultraviolet-induced malignant transformation *in vitro*, and in particular to investigate the role of ultraviolet-induced DNA repair processes with respect to the end point of transformation. Such experiments are in progress.

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Asbestos cytotoxicity in a long term macrophage-like cell culture

ASBESTOS is well documented as a carcinogen and cocarcinogen in man and experimental animals, and malignancy may appear after exposure to small amounts. In people who have worked with asbestos insulation there has often been a lag of 20 yr between the first exposure and the development of neoplasia. Among these workers a high incidence of carcinoma of the lung and a high frequency of the rare neoplasm mesothelioma has been reported¹. The risk of death from lung cancer is 92 times greater for an asbestos worker who smokes than for someone who is statistically comparable and neither smokes nor works with asbestos. The additive effect of smoking and exposure to asbestos is much greater than that of either factor alone, indicating a cocarcinogenic effect². Exposure to asbestos is not confined to the occupational environment, for asbestos is present in the air of 49 cities in the USA, in some talcum powders and gypsum spackles and in about 400 industrial products³. As the initial *in vivo* response to asbestos involves uptake by host macrophages⁴, we have quantified the effects of standard Union Internationale contre le Cancer (UICC) reference samples of amosite and chrysotile B asbestos *in vitro* on the P388D1 line of malignant cells. These cells have macrophage-like characteristics⁵, but, unlike fresh culture systems, they are capable of reproduction and make long term experimentation possible. We found that asbestos toxicity could be measured as a function of the percentage of cells associated with fibres. Chrysotile was more toxic per unit weight than amosite because it had more fibres per unit weight that could become associated with cells.

Available information on the basic biological effects of asbestos in macrophage cell culture systems has been

reviewed recently⁶. In previous studies fresh peritoneal cultures of hamster, mouse and guinea pig macrophages were exposed to samples of amosite and chrysotile⁷⁻¹². The resulting cytotoxicity was thought to be due to the interaction of asbestos fibres with plasma membrane, and was lessened by increasing the amount of serum. A delayed cytotoxicity was demonstrated by the release of increased amounts of acid phosphatase and β -glucuronidase into the media. In all these cases chrysotile was more toxic per unit weight than amosite.

The crystalline properties of asbestos enabled us to use rectified polarisation optics to detect small birefringent particles of asbestos fibres associated with cells in culture. We used UICC standard reference samples of amosite and chrysotile B, which have been characterised extensively¹³⁻¹⁵. The external surface area of the amosite is $3.3 \text{ m}^2 \text{ g}^{-1}$ while that of the chrysotile is $4.9 \text{ m}^2 \text{ g}^{-1}$. In the optical microscope fibres of amosite and chrysotile vary from 2 to $60 \mu\text{m}$ long. Chrysotile has 6% more fibres $>4 \mu\text{m}$ than amosite. Previous studies have shown that mouse peritoneal macrophages will phagocytose completely asbestos fibres less than $5 \mu\text{m}$ long. Fibres between 5 and $20 \mu\text{m}$ long are sometimes ingested completely while beyond this length complete phagocytosis is not possible.

With both amosite and chrysotile asbestos we found dose-response cytotoxicity. All cultures which reached a nadir of

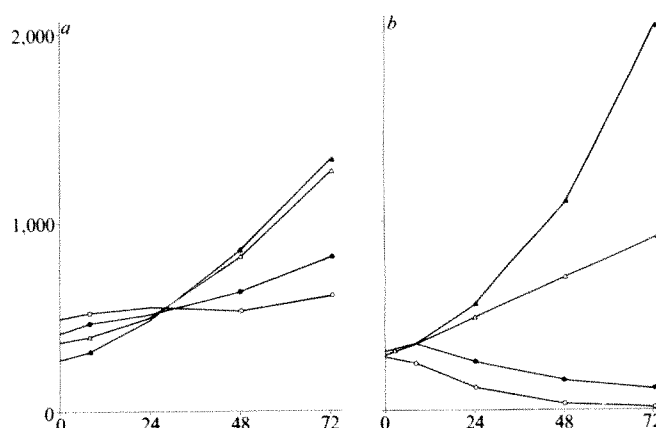


Fig. 1 Growth curves showing the number of P388D1 cells after exposure to amosite (a) and chrysotile (b). Four 250-ml Falcon tissue culture flasks were each inoculated with 10 ml of 4×10^5 viable P388D1 cells per ml of passage number 37 for the amosite and passage number 41 for the chrysotile. Fischer's media for leukaemic cells of mice plus 10% foetal calf serum with a total volume of 10 ml per flask was used. The flasks were incubated stationary at 37°C in 5% CO_2 in air. After 24 h the medium was changed in all flasks and steam-sterilised asbestos was added to three of the flasks in concentrations of 10, 50 and $100 \mu\text{g ml}^{-1}$ with a total volume of 10 ml per flask. The fourth flask was used as a normal uninoculated control. Medium containing asbestos was left in contact with the cells for 48 h and then removed. Throughout the experiment medium without asbestos was used for complete changes of medium on a regular basis, generally every 2 d. To standardise observations of the culture fields, paper tape with 1-mm holes was taped randomly to the outside of the flasks. There were 10 observation holes for each flask of cells. Using an inverted Nikon microscope fitted with bright field phase objectives and $\times 10$ eyepieces, each field was first focused and observed with a $\times 10$ objective to maintain uniformity of field position, and photographs were then taken through the $\times 20$ objective. This allowed repeated photographs of the same field of cells. Observations and photographs were made immediately before (time zero) the addition of asbestos-containing medium, and then after 8, 24, 48 and 72 h. Each of the 10 fields on each flask was photographed at each time point, and the total of morphologically identifiable cells per photograph was counted in a standardised manner by one observer. Growth curves were derived from these counts. ▲, normal; △, asbestos ($10 \mu\text{g ml}^{-1}$); ●, $50 \mu\text{g ml}^{-1}$; ○, $100 \mu\text{g ml}^{-1}$. At all doses the standard error of the mean (s.e.m.) was less than 15%, except for chrysotile at $50 \mu\text{g ml}^{-1}$ and $100 \mu\text{g ml}^{-1}$ at 48 and 72 h, when it was 33%.

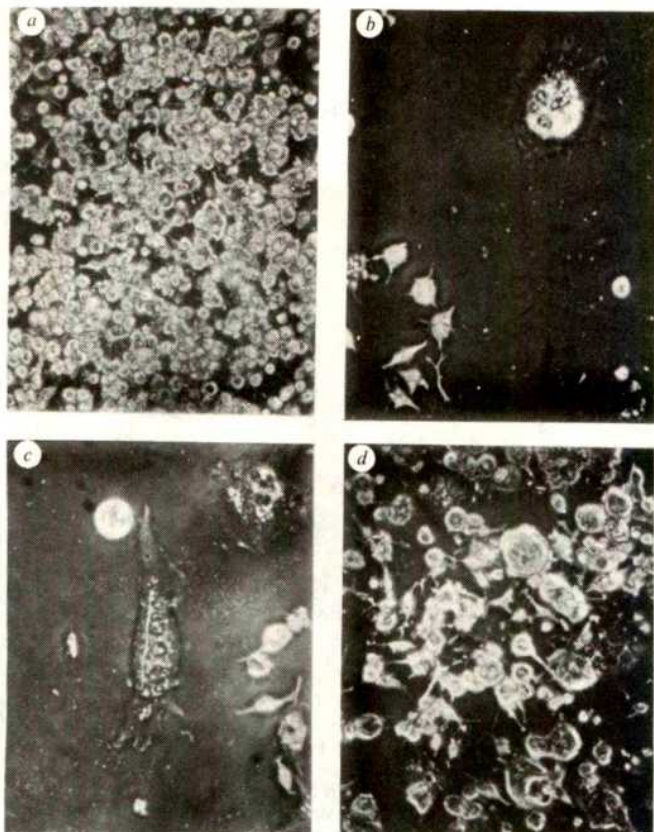


Fig. 2 Morphology of the P388D1 cells after 8–10 d exposure to UICC samples of amosite and chrysotile B asbestos ($50 \mu\text{g ml}^{-1}$). *a*, Normal control after 10 d. Note the large number of small round cells. *b*, After 10 d of exposure to chrysotile ($50 \mu\text{g ml}^{-1}$). Cells are scarce and have changed in size, accompanied by the 'sunburst' morphology. *c*, After 8 d of exposure to chrysotile ($50 \mu\text{g ml}^{-1}$). Note the later conformation of this cell to the shape of the cell-associated fibre. *d*, After 10 d of exposure to amosite ($50 \mu\text{g ml}^{-1}$). The number of cells present and their size morphology should be compared with those of the chrysotile (Fig. 2). ($\times 200$).

growth 72 h after exposure to asbestos maintained the ability to become confluent again within a time proportional to the initial decrease in cell number. Morphological changes occurred after the initial exposure to asbestos. With amosite these included increasing size, numerous thin dendritic projections and an initial change of configuration of a cell conforming to the shape and size of the particles with which the cell was associated. In contrast, although cells exposed to chrysotile did not appear to change shape initially to conform with that of the particles, they often did so later. They soon became larger than normal cells or those exposed to amosite, and took on a characteristic surface appearance with numerous short projections in a "sunburst" pattern.

Table 1 Effects of percentage of cell-associated fibres after 3 h on 72-h cell growth

Asbestos	Dilution ($\mu\text{g ml}^{-1}$)	Time (h)	% of cell-associated fibre (coverslips)	% of cell death at 72 h (bottles)
Amosite	10	3	36.9	4.7
Amosite	50	3	100	38.6
Amosite	100	3	100*	54.0
Chrysotile	10	3	96.4	55.0
Chrysotile	50	3	100†	94.3
Chrysotile	100	3	—‡	99.1

*More than one fibre per cell.

†Three or four fibres per cell.

‡A few cells left.

Untreated P388D1 cells had little birefringence except that of the mitotic spindle. Toxicity of both amosite and chrysotile was related to the percentage of cells with associated birefringent asbestos fibres (Table 1). For example, the similar percentage of cell-associated fibres present after 3 h with amosite at $50 \mu\text{g ml}^{-1}$ and chrysotile at $10 \mu\text{g ml}^{-1}$ correlated with similar percentages of cell death at 72 h. Asbestos fibres in the cultures were counted from photographs taken after 8 h of exposure: there were 378 fibres with the chrysotile, and 366 fibres with the amosite (concentrations as above). Therefore the difference in toxicity per unit weight of chrysotile and amosite relates to the number of fibres associated per P388D1 cell.



Fig. 3 Birefringence of P388D1 cells after 8 h of exposure to chrysotile ($100 \mu\text{g ml}^{-1}$). Coverslips (25 mm in diameter) in 2 ml of medium were inoculated with the same cells and the same concentrations of both types of asbestos. Observations and photographs were made with a Leitz polarisation microscope fitted with Nikon rectified objectives. Under a polarisation microscope the crystalline nature of asbestos produced increased contrast of black or white fibres against a grey background. The cell-associated fibres changed contrast at different compensator settings, indicating that the fibres were birefringent. A and P indicate vibration planes of analyser and polariser, respectively. This photograph was taken at a compensator setting 7° to the side of the background compensation position. Measurements were made of the percentage of cells with associated birefringent asbestos fibres at 3 and 12 h after addition of asbestos.

In our quantitative investigation of cytotoxicity we found asbestos to be as cytotoxic for malignant P388D1 cells as it has been reported to be for non-malignant fresh macrophage culture systems. Therefore these malignant cells would make a good model system for the study of long term effects of asbestos-cell interactions. This *in vitro* system should make it possible to gain some insight into the long *in vivo* macrophage effect in the interim between asbestos exposure and the development of neoplasia.

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Effect of a phorbol ester on a transformation-sensitive surface protein of chick fibroblasts

PMA (phorbol myristate acetate) is the most active of a class of compounds present in croton oil which act as tumour promoters¹⁻³. While PMA possesses little carcinogenicity in its own right, its repeated application to mouse skin causes tumour induction in animals treated up to several months previously with a single subthreshold dose of a carcinogen. If the order of treatments is reversed, that is, if PMA treatment precedes that with the carcinogen, then no tumours are induced. Studies both with mouse skin and with cells in tissue culture indicate substantial effects of PMA on cell behaviour and biochemistry. Many of these effects such as alteration of phospholipid synthesis⁴ or stimulation of Na⁺, K⁺-ATPase and 5'-nucleotidase⁵ relate to the cell membrane. We report here that PMA alters the cell surface protein composition of chick embryo fibroblasts, causing a decrease in the major surface protein LETS (molecular weight 250,000). Previous work by several laboratories has indicated that LETS, which is present on the surface of fibroblasts from a variety of species, either decreases or disappears upon transformation⁶⁻¹¹. Although LETS is highly sensitive to cleavage by proteases such as plasmin¹², its mechanism of loss following transformation remains to be clarified.

We find that the addition of PMA to exponentially growing cultures of secondary chick embryo fibroblasts led to alterations in the pattern of iodinated surface proteins fractionated on sodium dodecyl sulphate (SDS)-polyacrylamide gels. Several bands were decreased in amount,

while at least one new band appeared. Most striking was a reduction in the amount of LETS. After incubation of exponentially growing chick embryo fibroblasts in the presence of PMA for 3 d, the amount of radioactivity in LETS per cell was decreased to 12% of the control value (Fig. 1). The magnitude of the decrease in LETS depends on the basis used for comparison. PMA had little effect under the usual experimental conditions on cell number per plate,

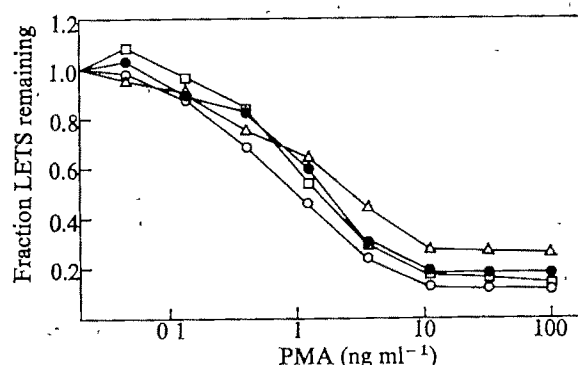


Fig. 1 Effect of PMA on LETS. Secondary chick embryo fibroblasts were plated at 1×10^5 per 60 mm dish in MEM containing 10% tryptose phosphate broth, 5% heat-inactivated chicken serum, penicillin (75 u ml^{-1}) and streptomycin (50 µg ml^{-1}). PMA (Consolidated Midland) dissolved in DMSO was added to each plate to give the indicated final concentrations. The concentration of DMSO in all plates was constant, 0.10%. After 3 d, the cells were iodinated, the proteins fractionated by SDS-polyacrylamide gel electrophoresis on 5% gels, and the amounts of LETS determined as described¹³. The "fraction LETS remaining" gives the c.p.m. in LETS from the plate with PMA per c.p.m. in LETS from the control plate with DMSO. The data were normalised where indicated as follows: (○) Fraction remaining, normalised to cell number; (●) Fraction remaining, normalised to the total surface label migrating on the SDS-polyacrylamide gels with mobility between that of LETS and the marker dye; (△) Fraction remaining, normalised to total protein. All points are the average of duplicate measurements. 1.0 = 31,000 c.p.m. in LETS/gel sample.

total iodine label incorporated, or incorporated iodine label migrating on SDS-polyacrylamide gels with a mobility between that of LETS and of the marker dye bromophenol blue. The decrease in the amount of LETS relative to that of controls was thus similar when normalised to any of these bases. While the amount of LETS relative to other surface label is probably the most relevant value, the volume of chick embryo fibroblasts treated with PMA decreased by a factor of two (manuscript in preparation) and the amount of protein per cell decreased comparably. Consequently, the amount of LETS was reduced to 25% of that in the untreated control when expressed per μg of cell protein.

The growth state of cells in culture is often a significant variable affecting cell surface properties. The observed decrease in LETS caused by PMA probably cannot be explained by such an effect. In the conditions of Fig. 1, the addition of PMA had little effect ($\pm 20\%$) on absolute cell number per plate, on the rate of increase in cell number, and on incorporation of ³H-thymidine per cell.

The amount of PMA required for the half-maximal effect on LETS was $1-2 \text{ ng ml}^{-1}$. This value corresponds to a concentration of $1.5-3 \times 10^{-8} \text{ M}$. Increase in the concentration of PMA between 10 and 100 ng ml^{-1} had no further effect on LETS.

The decrease in LETS following addition of PMA proceeded over a 3-d time course. If cells were replated at this time into fresh PMA-containing medium, this low level was maintained. This loss was slower than that reported after transformation of chick embryo fibroblasts by Rous sarcoma virus⁴. Likewise, full expression of the effects of

PMA on LETS took much longer than its effects on some other properties, for example stimulation of ^3H -deoxyglucose uptake in chick embryo fibroblasts (4 h) or stimulation of cyclic GMP in 3T3 cells (1 min)¹³. It should be noted that the kinetics of loss of LETS after addition of PMA refer to the total amount of LETS remaining on the cell surface. The rapidity of the response to addition of PMA in the production and/or attachment of new LETS remains to be established.

The loss of LETS brought about by PMA was not an irreversible process. Cells treated with PMA regained LETS within 3 d of trypsinisation and replating in medium lacking PMA. PMA was thus not inducing permanent transformation of the cells. The 3-d time course for reappearance of LETS represents an upper limit on the recovery time. Since PMA is highly lipid soluble¹⁴, the possible role of residual cell-bound PMA in delaying recovery of LETS is not known. For comparison, upon shift to the non-permissive temperature of chick embryo fibroblasts transformed by a temperature sensitive mutant of Rous sarcoma virus, LETS returned to normal levels with a 1-d time course⁸.

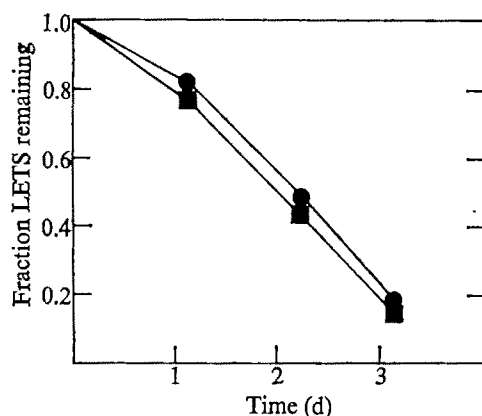


Fig. 2 Time course of decrease in LETS after addition of PMA. Cells were plated and treated with PMA as described in the legend to Fig. 1, except that the initial plating densities were varied so that the cell densities at the time of iodination were similar. At the indicated times, plates treated with DMSO or PMA were iodinated and LETS determined. Fraction LETS remaining has been normalised to the total surface label migrating on the SDS-polyacrylamide gel with a mobility between that of LETS and the marker dye. (●) PMA, 3 ng ml⁻¹. (■) PMA, 10 ng ml⁻¹.

The direct cause for the decrease in LETS upon treatment of the chick embryo fibroblasts with PMA is not known. For the loss of LETS induced by viral transformation, the following possibilities have been considered: (1) failure of the cells to synthesise LETS, (2) failure of LETS to be properly inserted and bound to the membrane, or (3) proteolytic cleavage of LETS from the membrane after binding. With regard to an earlier level of regulation, the possibility that PMA does not affect LETS *per se* but regulates some intermediate property, which itself determines the level of LETS, deserves consideration. In either case, the understanding of the mode of action of PMA on LETS is of particular interest in that it provides a new approach and may potentially provide new insight into the control of this same surface component by Rous sarcoma virus.

A number of the effects of PMA on cells resemble those induced by transformation. These effects include (1) decrease in LETS, (2) increase in plasminogen activator¹⁵, (3) release from contact inhibition¹⁶, (4) increase in cyclic GMP¹³ and decrease in cyclic AMP concentrations¹⁷, and (5) reduction in cell adhesion, loss of orientation on the

plate and alteration in morphology¹⁸. Caution must be exercised in extrapolating these results since a number of these changes have been observed in systems other than those, for example the mouse epidermis and certain cell culture systems¹⁹, where the promoting effects of PMA have been characterised. Nonetheless, the parallels between the effects on cells of PMA and of transformation are consistent with the hypothesis that tumour promoters might function by causing the conversion of cells from a normal phenotype to one more resembling the transformed state, thus facilitating expression of the genotype of latent tumour cells.

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Kinetics of lymphocyte stimulation by concanavalin A

AN important problem pertinent to the mechanism of lymphocyte stimulation with a nonspecific mitogen is how long lymphocytes must be exposed to the mitogen to attain maximal stimulation. In the case of the stimulation of human and murine lymphocytes by concanavalin A (con A), the cells are irreversibly committed to stimulation if the lectin is present on the cell membranes for some 18-20 h after the start of the culture¹⁻³. Here we report studies on the kinetics of the stimulation of mouse splenic lymphocytes by con A. Our data indicate that two pulses of con A (0-3 h and 15-18 h after the start of the culture) can stimulate the cells to the same degree as the continuous exposure of the cells to con A for 18 h, suggesting that two signals are necessary for the con A stimulation of lymphocytes. We also show that the first signal is possibly given by the influx of Ca²⁺.

As shown in Fig. 1, when con A on the membranes of mouse splenic lymphocytes was removed by washing with methyl α -D-mannopyranoside (α -MM) at various times after the start of the culture, the response of the cells as measured by ^3H -thymidine incorporation did not increase linearly with the time of the exposure to con A, but it increased rapidly when the cells were exposed to con A for periods longer

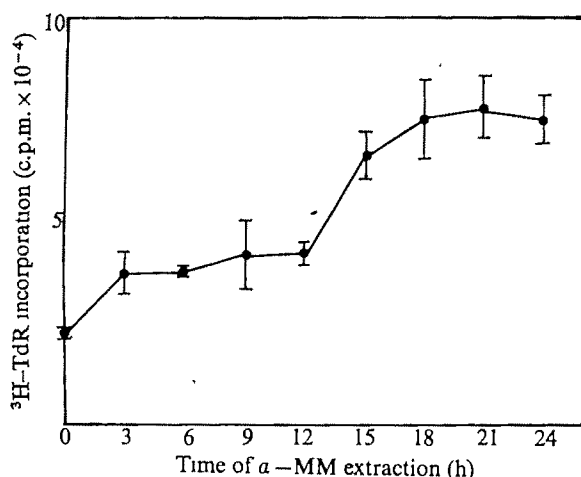


Fig. 1 The effect of con A removal at different times after the start of the culture on the ³H-thymidine incorporation by con A-stimulated mouse splenic lymphocytes. Splenic lymphocytes (2×10^6 cells ml^{-1}) purified from C3H/He mice by Ficoll-Urografin density gradient centrifugation⁶ were incubated in a silicone-coated test tube (1×10 cm) with con A ($4 \mu\text{g ml}^{-1}$) in 1.0 ml of RPMI 1640 medium supplemented with 10% foetal calf serum, 2 mM glutamine and kanamycin ($60 \mu\text{g ml}^{-1}$) at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. At different times, cultures were washed in the culture medium and resuspended in 1.0 ml of 0.05 M α -MM in the culture medium. After 10 min, the cells were collected by centrifugation (300g, 5 min) and resuspended in 1.0 ml of the culture medium. Incorporation of ³H-thymidine ($1.0 \mu\text{Ci}$ per culture; specific activity 23 Ci mmol^{-1}) between 28 and 42 h after the start of the culture was measured by the method described previously⁶. Each point represents the average of three cultures and vertical bars represent ± 1 s.e.m. As control experiments, the incorporation of ³H-thymidine of an unstimulated culture not treated with α -MM (\square) and of a culture continuously exposed to con A for 42 h (\blacksquare) was shown.

than 12 h. No inhibition was observed when con A was removed at times later than some 18–20 h after the initial binding of the lectin.

We then compared the response after various pulsed exposures to con A with that after the continuous exposure to the lectin for 18 h. Within experimental error, two successive 3-h exposures (0–3 and 15–18 h) separated by an

interval of 12 h yielded the same level of response as continuous exposure for 18 h (Table 1), whereas, when the cells were pulsed with con A only for 3–6 h at the beginning of the culture, or for 3 h between 15 and 18 h, the response was only 25–40% of that seen with an 18-h exposure to con A. These observations were further confirmed by the comparison of the kinetics of DNA synthesis among the cultures which were pulsed with con A at various times. The culture pulsed twice (0–3 and 15–18 h) with con A gave a similar DNA synthesis profile to that of the culture in which the cells were in contact with con A continuously for 18 h (Fig. 2), reaching maximal incorporation of ³H-thymidine by 40 h after the initial binding of con A to the cell membranes. In the other experiments, we shortened the length of the two-pulsed exposure to con A to 1 or 2 h each, but the response was found to be 70–80% of that seen with two 3-h exposures. The requirement of the presence of mitogen on the cell surface at two different stages—at the beginning of culture and at the period just before the onset of DNA synthesis—for maximal lymphocyte stimulation has also been suggested by Weber *et al.*⁴

Since recent investigations have shown that the enhanced uptake of Ca^{2+} is one of the common events which occur within minutes of the binding of mitogen to lymphocytes^{7,8}, we replaced the first exposure to con A (0–3 h) with treatment with calcium ionophore A23187 which has also been shown to be mitogenic to lymphocytes^{9–11}. The data in Table 1 reveal that the pulsed treatment of lymphocytes with A23187 for 3 h at the beginning of the culture followed by a 3-h exposure to con A between 15 and 18 h after the start of the culture is equally effective in the activation of lymphocytes as the two pulses of con A (0–3 and 15–18 h). The addition of EGTA (5×10^{-4} M) to the culture medium with A23187 at the beginning of the culture, however, reduced the stimulation to 60–70%. Furthermore, the response of the culture pulsed with con A first (0–3 h) and then with A23187 (15–18 h) was much smaller than that of the culture pulsed with A23187 first and with con A later.

These results suggest that the Ca^{2+} influx into the cell mediated by ionophore or induced by the mitogenic lectin on the cell membranes possibly plays an important part in the triggering of lymphocyte stimulation as the first signal. In view of the recent findings that guanylate cyclase activity of lymphocytes is dependent on the intracellular

Table 1 Effect of pulsed exposure of lymphocytes to con A and calcium ionophore A23187

Time (h)						³ H-Thymidine incorporation (c.p.m.)
0	3	9	12	15	18	
—C—				—C—		11,219 ± 391
—C—		—C—		—C—		17,090 ± 568
—C—				—C—		21,360 ± 3,235
—C—		—C—		—C—		37,038 ± 3,947
—A—				—A—		34,035 ± 88
—A—				—A—		11,492 ± 178
—A—				—A—		14,755 ± 866
—A—				—A—		15,318 ± 1,211
—A—		—A—		—A—		16,452 ± 1,354
—C—				—A—		21,852 ± 1,393
—A—				—C—		36,465 ± 4,390
—A—				—C—		29,259 ± 2,499
Cell control						9,398 ± 1,975

Mouse splenic lymphocytes were cultured with con A ($4 \mu\text{g ml}^{-1}$) or with A23187 ($0.05 \mu\text{g ml}^{-1}$) as described in the legend of Fig. 1. For the removal of con A and A23187, the following washing procedure was carried out in both cases. At an appropriate time after the start of the culture, cells were washed once in the culture medium and resuspended in 1.0 ml of 0.05 M α -MM in the culture medium. After 10 min, the cells were collected by centrifugation (300g, 5 min) and resuspended in 1.0 ml of the culture medium. Incorporation of ³H-thymidine between 39 and 42 h after the start of the culture was measured by the method previously described⁶. Each value represents the mean \pm s.e.m. of three cultures. The horizontal bars represent the time during which con A (C) or A23187 (A) was present.

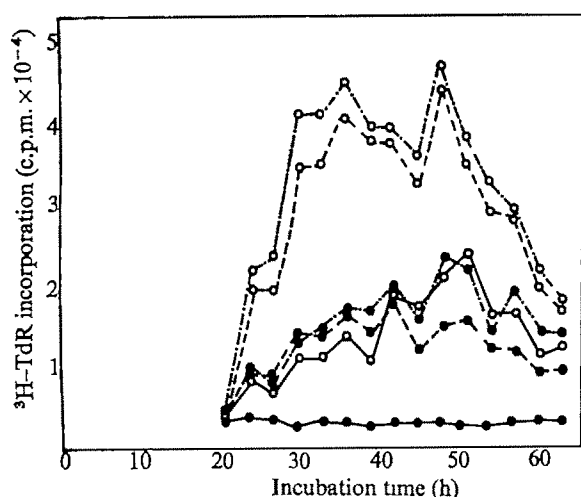


Fig. 2 The effect of length of exposure to con A on the kinetics of DNA synthesis in mouse splenic lymphocyte cultures. Culture of lymphocytes and the removal of con A from each culture were performed as described in the legend of Fig. 1. ○, Control cultures without con A. Periods for which the cultures were incubated with con A ($4 \mu\text{g ml}^{-1}$). □, 0–3; ■, 0–6; ●, 15–18; ▲, 0–3+15–18; △, 0–18 h. DNA synthesis was measured with a terminal 3-h pulse of ^3H -thymidine ($2.5 \mu\text{Ci}$ per culture). Each point represents the average of three cultures.

level of calcium ion¹² and microtubule function is controlled by the local concentration of Ca^{2+} in the cell cytoplasm^{13,14}, the mitogen-induced influx of Ca^{2+} into lymphocytes triggers the stimulation of the cells possibly by the elevation of cyclic GMP level in the cells and/or the functional alteration of microtubular assembly which modulates the topography of cell-surface receptors. After approximately 12–15 h from the influx of the first signal, the cells attain a preactivated state, and these then proceed to the DNA synthetic period (S phase) if the second signal is provided by the presence of mitogen on the cell membrane. It may, however, be necessary to estimate actual numbers of responding cells during the course of the exposure to con A to validate this assumption further. Investigation of the nature of the second signal and its mechanism of action should enable us to elucidate the triggering mechanism of lymphocyte activation.

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Rat platelets aggregate in the absence of endogenous precursors of prostaglandin endoperoxides

THE prostaglandin (PG) endoperoxides, PGG_2 and PGH_2 , are thought to have a function in human platelet aggregation, since they are formed during thrombin-induced aggregation¹. They induce aggregation on addition to platelet-rich plasma (PRP), either directly or after conversion to thromboxane A_2 (TXA_2)². TXA_2 (half life ~ 30 s) is formed *in vitro* by a microsomal enzyme which is insensitive to inhibition by aspirin-like drugs^{3,4}. It is therefore distinct from the cyclo-oxygenase that converts arachidonic acid (AA) into PG endoperoxides. AA, after incorporation into membrane phospholipids, is liberated during thrombin-induced aggregation and is then almost completely metabolised⁵ by the cyclo-oxygenase and AA-lipoxygenase⁶. Therefore, we tested platelets of animals that were deprived of AA, and still observed aggregation with collagen, indicating that PG endoperoxides and TXA_2 are not essential for aggregation.

Feeding mammals with an essential fatty acid-deficient (EFAD) diet, lacking linoleic acid ($18:2$, $n-6$), results in a decrease of *bis*-homo- γ -linolenic acid ($20:3$, $n-6$) and AA ($20:4$, $n-6$), both PG precursors. The part played by AA in membrane phospholipids is replaced by eicosatrienoic acid ($20:3$, $n-9$), which is not a substrate⁷ but a competitive inhibitor⁸ of the cyclo-oxygenase. Pregnant rats (Wistar, TNO, Zeist, The Netherlands) were placed on a diet containing 4% hydrogenated cocofat (Hope Farms), 5 d before the expected day of delivery⁹. After weaning, the newborn rats were kept on EFAD food; controls received 3.5% of their calories as linoleic acid. Apart from reduced growth¹⁰, the EFAD condition of the rats was clearly demonstrated by fatty acid analysis of erythrocytes (Table 1). A marked reduction of the linoleic acid family ($18:2$, $n-6$; $20:3$, $n-6$; $20:4$, $n-6$) was observed in favour of the oleic acid ($18:1$, $n-9$) family. The criterion of Holman¹⁰ for EFA deficiency—a ratio between eicosatrienoic acid ($20:3$, $n-9$) and AA ($20:4$, $n-6$) greater than 0.4—was amply exceeded.

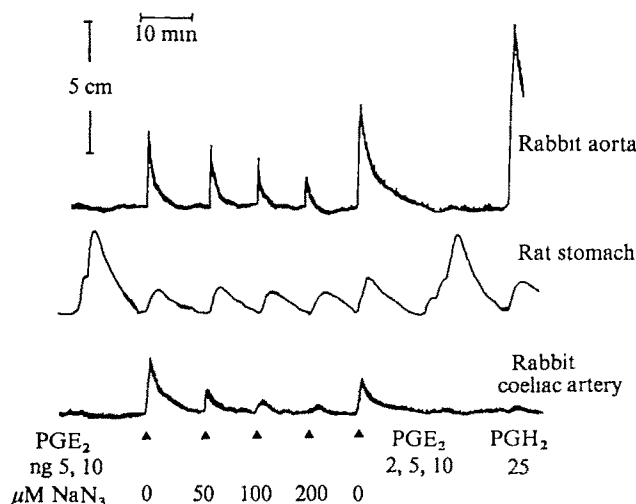


Fig. 1 Differences in bioassay of rat platelet RCS and PGH_2 . The cascade, with rabbit aorta, rat stomach and rabbit coeliac artery strips, was superfused with a Krebs' solution (2.5 ml min^{-1} , 37°C) containing a mixture¹¹ of antagonists to inactivate serotonin, histamine, acetylcholine and catecholamines and indomethacin ($3 \mu\text{M}$) to prevent PG synthesis by the tissues. Cumulative¹² doses of PGE_2 and PGH_2 in glucose-free Krebs' were superfused over the tissues. Samples (0.1 ml) of normal PRP, preincubated for 3 min at 37°C with different concentrations of NaN_3 , were superfused directly (▲) on the cascade, 1 min after addition of collagen ($40 \mu\text{g ml}^{-1}$). Preparation of PRP and aggregation was performed as described in Table 2.

Table 1 Fatty acid composition of total fat from normal and EFAD rat erythrocytes

Fatty acid	Normal % weight	EFAD % weight
16:0	29.8±1.3	28.9±0.9
18:0	20.0±1.5	17.5±2.1
18:1, <i>n</i> -9	10.2±1.1	19.8±0.7
18:2, <i>n</i> -6	7.6±0.7	0.8±0.04
20:3, <i>n</i> -6	0.2±0.04	<0.1
20:3, <i>n</i> -9	<0.1	8.7±0.9
20:4, <i>n</i> -6	18.6±1.7	4.5±0.6
<i>R</i> =20:3, <i>n</i> -9 20:4, <i>n</i> -6	<0.013±0.002	1.97±0.09

Heparinised blood was obtained by cardiac puncture from male rats (23–25 weeks old) under ether anaesthesia. After centrifugation (30 min, 950g) plasma and 'buffy coat' were removed and the erythrocytes were washed three times with 6 volumes of 10 mM EDTA in saline. Total fat of 5 ml packed cells was isolated²⁴ by two extractions with 19 volumes of chloroform-methanol (2:1). After saponification of 5 mg lipid, the fatty acids were extracted (pH=4) with pentane (3×25 ml), dried over anhydrous Na₂SO₄ and methyl esters were prepared in a fresh ethereal diazomethane solution (nitrogen atmosphere). The fatty acid pattern was determined with a Hewlett Packard 5750 gas chromatograph, equipped with a 4 foot×3-mm column (3% EGSS-X on gaschrom Q; Chrompack, The Netherlands) and a flame-ionisation detector. The equivalent chain length values were compared with those of a mixture of standard fatty acids and with the data of Hofstetter²⁵. Weight percentages were determined by triangulation, and expressed as means (±s.e.) of four rats.

Aggregation induced by different stimuli was investigated in both normal and EFAD PRP (Table 2) and no significant differences in platelet number were observed *in vivo*¹¹ or in PRP. In rat platelets adenosine diphosphate (ADP) induces only primary aggregation¹³, which is probably independent of the PG-endoperoxide mechanisms¹³. Therefore, a supra-maximal dose of ADP (40 μM) was added after each aggregation, serving as control for the aggregatory capacity of each sample. ADP induced equal aggregation in normal and EFAD PRP, except at the lowest dose, when EFAD PRP aggregated less (56%) than normal PRP. Although electron microscopy did not reveal membrane differences, this result might reflect an altered membrane structure¹⁴.

The sensitivity of rat PRP towards the PG-endoperoxide PGH₂ seemed to be low. At low doses (from 500 ng ml⁻¹) PGH₂ only induced a minor shape change as seen from a small decrease in light transmission and diminished oscillations. With 1 μg ml⁻¹ a small transient aggregation was observed. Only small amounts of PGH₂ may enter the rat cells and therefore may not be converted to TXA₂ by the internal microsomal enzyme system. Preloading of PRP with a high concentration of linoleic acid, in order to eliminate binding of PGH₂ to albumin¹⁵, did not alter rat PRP responses. No significant differences were observed between EFAD and normal PRP (*P* > 0.05, analysis of variance) in any condition.

EFA deficiency did not alter the aggregating efficacy of high collagen doses, but inhibited almost completely aggregation by a dose giving half maximal responses in normal PRP. Since feedback inhibition by PGD₂, the most potent inhibitor of human platelet aggregation^{16,17}, is absent in rat platelets¹⁷, the diminished response from platelets with a low internal AA content might be caused by a rate-limiting production of PG-endoperoxides and TXA₂. To check this assumption the release of substances derived from AA and involved in aggregation was assessed directly on a cascade¹⁸ of isolated tissues¹⁹.

The substances formed by aggregating normal rat PRP, were differentiated by using a combination of rabbit aorta^{19,21}, rat stomach^{18,19} and rabbit coeliac artery²⁰ strips, in the presence of appropriate antagonists²¹ (Fig. 1). PRP, 1 min after addition of collagen (40 μg ml⁻¹) contracted all tissues. All smooth muscle-stimulating activities, including rabbit aorta contracting substance¹⁹ (RCS) were lipid soluble, because they were removed after passage through a small Amberlite XAD-2

column, as shown previously²¹. Rabbit coeliac artery has been reported to contract to TXA₂ and to show relaxation towards PGE₂ and PGH₂ (ref. 20). The latter observation was not confirmed and small contractions were observed with higher doses of PGH₂. TXA₂ may be generated by aggregating PRP since rabbit coeliac artery shows great sensitivity towards the contractile activity of TXA₂ (ref. 20).

Rabbit aorta is sensitive towards PGH₂, but TXA₂ is far more potent than the PG-endoperoxides^{3,4,20}. Although comparison of sets of rabbit aorta and coeliac artery responses towards control PRP (0 μM NaN₃) indicates that a TXA₂-PG-endoperoxide balance seems to exist, the main component of RCS is probably TXA₂. This was confirmed by the half life of RCS at 37 °C, 32±6 s (*n* = 5), following removal of platelets by quick filtration through a Millipore filter to avoid new TXA₂ formation. The half life of PGH₂, stirred in platelet-poor plasma (900 r.p.m., 37 °C), was 125±42 s (*n* = 8). These half lives were estimated from linear plots of the logarithms of rabbit aorta contractions at different times. Finally, inhibition of RCS by low doses of NaN₃, without altering the rat stomach contractions, again indicated that RCS differs from PG-endoperoxides. Rat stomach is stimulated by PGE₂, to a lesser extent by PG-endoperoxides²¹ and PGD₂, but hardly at all by TXA₂ (refs 3, 4, 20). The mechanism of azide interference with TXA₂, also demonstrated²² in rabbit PRP aggregated by AA, remains obscure. Possible explanations are inactivation of the TXA₂-forming microsomal system or an enhanced conversion of TXA₂ to the N₃ analogue of the inactive TXB₂ (ref. 2). As expected, no differences were observed in IC₅₀ values for TXA₂ and PGE inhibition by the cyclo-oxygenase inhibitors indomethacin and eicosatetraynoic acid, because both act at a stage before TXA₂ formation (unpublished results).

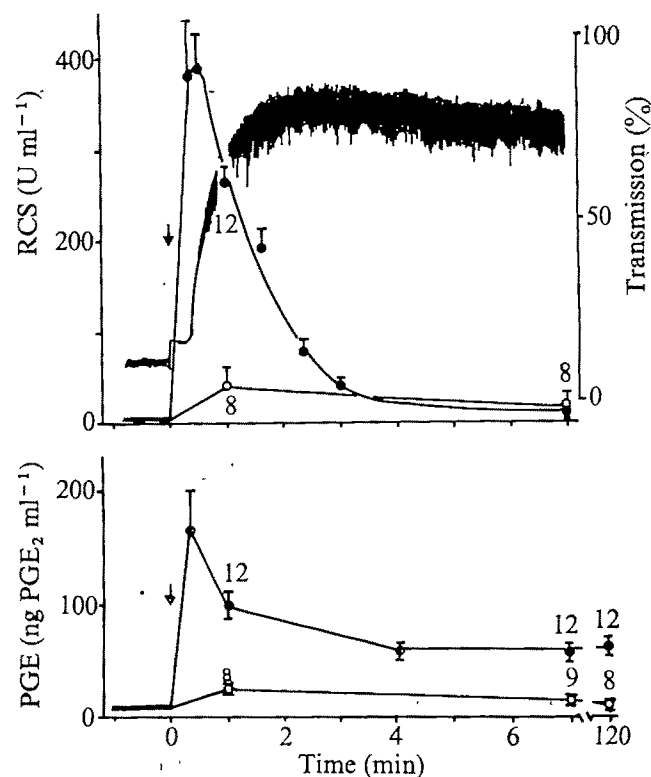


Fig. 2 Time courses of aggregation and generation of RCS (mainly TXA₂), PG endoperoxides and PGE by aggregating normal (●) and EFAD (○) PRP. Samples (0.1 ml) of rat PRP, aggregating after addition of 40 μg ml⁻¹ collagen (arrow) were superfused directly on rabbit aorta, and rat stomach strips (see legend Fig. 1). The upper part shows the changes in light transmission during aggregation of normal PRP together with release of RCS (expressed in units, 1 unit = 1 ng PGH₂ ml⁻¹). The lower part shows rat stomach contractions, calibrated with PGE₂. Each value is the mean (±s.e.) of four experiments, unless indicated otherwise.

Table 2 Comparison between the aggregatory capacity of normal and EFAD platelet-rich plasma (PRP)

	Normal PRP	EFAD PRP
Platelet count 10 ⁸ cells µl ⁻¹	618±38 (5)	729±31 (5)
Aggregation inducer	% Aggregation	% Aggregation
ADP 2.50 µM	95.7±2.6 (5)	90.8±1.5 (5)
0.80 µM	76.9±5.4 (5)	75.7±3.9 (5)
0.25 µM	25.9±3.0 (5)	14.5±2.4 (5)*
PGH ₂ 1 µg ml ⁻¹	25.0±5.5 (4)	17.9±4.0 (3)
Linoleic acid (10 mM)+ PGH ₂ 1 µg ml ⁻¹	14.7±4.3 (3)	15.5±2.7 (3)
Collagen 40 µg ml ⁻¹	95.2±1.6 (5)	95.3±1.6 (5)
4 µg ml ⁻¹	53.6±10.3 (5)	3.1±1.5 (5)†

PRP was collected after centrifugation (15 min, 200g, room temperature) of heparinised blood (see Table 1) and diluted with platelet-poor plasma (30 min, 950g) to A 602 nm/1 cm=0.9. PRP was prepared daily, avoiding any glass contact and stored at 0 °C. The platelet number was determined with a Coulter counter (Model A). PRP (200 µl) was preincubated (3 min, 900 r.p.m., 37 °C) with 25 µl Tyrode (glucose and Ca²⁺-free) or with 25 µl Tyrode containing linoleic acid. Aggregation was started by addition of 25 µl inducer and maximal increase in light transmission was measured (Payton Single Channel Module, Payton Ass. Inc., Buffalo, New York). Results are expressed as percentages of maximal increase with 40 µM ADP, added in 10 µl 5 min after starting aggregation. Collagen (Bovine achilles tendon, Sigma, final concentration 400 µg ml⁻¹) was suspended in 18 mM acetic acid. PGH₂ was stored at -70 °C in diethylether and after evaporation, it was immediately dissolved in Tyrode and tested. ADP (Boehringer) and linoleic acid (Merck) were also dissolved in Tyrode. The values are the means (± s.e.) of the indicated number of experiments.

* $P < 0.05$, † $P < 0.01$, Wilcoxon test, two-sided.

Release of TXA₂, PG-endoperoxides and PGE by normal and EFAD PRP, together with an aggregation pattern of normal PRP, is shown in Fig. 2. Although RCS is mainly TXA₂, the rabbit aorta strips were calibrated with PGH₂. No activity was detectable during preincubation. At 20 s after collagen addition, when aggregation is starting after a lag period, TXA₂ was almost maximally generated by normal PRP. TXA₂ concentration was practically unaltered at 30 s, when light transmission increased steeply. At 1 min, however, when aggregation is completed for 75%, TXA₂ is already disappearing and when maximal increase in light transmission, together with oscillations due to aggregates, was observed (3 min), hardly any TXA₂ was detectable. EFAD PRP generated only 16% of the TXA₂ released by normal PRP 1 min after collagen, but aggregation of EFAD PRP was unaltered with this high collagen dose (also shown in Table 2). PGE formation showed the same pattern, with the exception that a residual activity was observed which was stable for up to 2 h after aggregation. This activity behaved like PGE after extraction, column chromatography and differential bioassay on rat stomach strip and rat colon (H. B. and I. L. B., in preparation). Since TXA₂ is not spasmogenic on rat stomach³, the initial peak of activity was probably composed mainly of PG-endoperoxides. These compounds degrade to PGE₂ (ref. 23) and other catabolites. Again EFAD PRP generated less PGE (20% or less when assessed at 7 min or later) and less unstable PG-endoperoxide-like activity (24%, 1 min) than normal PRP. The decreased formation of TXA₂ and PGE by EFAD platelets was not a result of decreased cyclo-oxygenase activity. During AA (0.325 mM) induced aggregation PG-endoperoxide-like activity (1 min) and PGE (7 min) were formed in equal amounts by normal and EFAD PRP.

Although this differential bioassay is less accurate than gas-liquid chromatography-mass spectrometry of degradation products of TXA₂ and PG-endoperoxides, it gives a dynamic picture of the presence of separate biologically active substances during platelet aggregation. These data, obtained with platelets in a more physiological environment than in other studies^{3,5}, indicate that the capacity of platelets to aggregate with threshold

doses of collagen is dependent on the availability of endogenous AA. Decreased generation of TXA₂ and PG-endoperoxides coincides with diminished aggregation by EFAD platelets, although their sensitivity towards PG-endoperoxides and their cyclo-oxygenase activity are unchanged. With high collagen concentrations the PG-endoperoxide generating system is replaced by other mechanisms because a reduction of endogenous AA did not prevent platelet aggregation. Finally, specific inhibition of TXA₂ formation, as shown with sodium azide, might provide opportunities to reduce platelet aggregation without affecting the formation of PGD₂, which has strong anti-thrombotic effects in human platelets.

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Stimulation of 'irritant' receptors and afferent C-fibres in the lungs by prostaglandins

The lungs are among the many organs that generate, release and destroy prostaglandins (PGs)¹. It has been suggested that PGs act as 'local hormones'², so that prostaglandins E₁ and E₂, which relax the smooth muscle of bronchi and pulmonary blood vessels in many species, and F_{2α}, which contracts it, may be implicated in normal regulation of airway and pulmonary vascular calibre³. PGF_{2α} has been proposed as a causal factor in asthma⁴. The bronchoconstrictor effect of PGF_{2α} is reduced by atropine, hence a reflex component may be involved⁵. Cough and airway irritation have been reported in clinical trials of PGE₁ and PGE₂ as bronchodilators in the treatment of asthma^{6,7}. Thus PGs may stimulate afferent nerve endings in the lungs, and there has been speculation as to which endings are involved. We have recorded impulse activity from rapidly-adapting pulmonary stretch ('irritant') receptors^{8,9} and afferent C-fibre endings^{10,11} in the lungs of anaesthetised

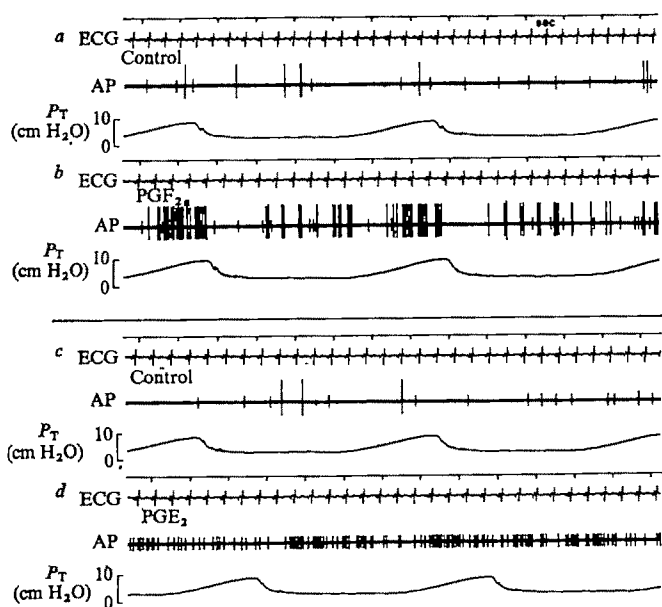


Fig. 1 Comparison of the effects of $\text{PGF}_{2\alpha}$ and PGE_2 on an 'irritant' receptor (large spikes) and a C-fibre ending (small spikes). Both endings were located in the lower lobe of the left lung. Conduction velocity was 27.2 m s^{-1} in the 'irritant' fibre, and 1.3 m s^{-1} in the C-fibre. *a*, Before, and *b*, 16 s after injection of $\text{PGF}_{2\alpha}$ ($4 \mu\text{g kg}^{-1}$) into the right atrium. Interval of 6 min between *b* and *c*. *c*, Before, and *d*, 42 s after injection of PGE_2 ($20 \mu\text{g kg}^{-1}$) into the right atrium. From above downwards in each record: 1 s time trace; ECG, electrocardiogram; AP, action potentials recorded from an afferent filament of the left vagus nerve; P_T , tracheal pressure (upstroke representing inflation).

dogs. When $\text{PGF}_{2\alpha}$ was injected into the right atrium, lung 'irritant' receptors were strongly stimulated. Injection of PGE_2 , by contrast, caused marked and prolonged stimulation of lung C-fibres.

We recorded impulses from fine strands (containing one, or at most two, active fibres) of the cervical vagus nerve in dogs anaesthetised with Dial-Nembutal. The two types of lung afferent were identified by their patterns of discharge, by their responses to various procedures (for example hyperinflation of the lungs; injection of chemicals known to stimulate them), and by the conduction velocity of their fibres⁸⁻¹¹. The chest was open so that we could determine by direct mechanical stimulation the location of the ending whose impulses we had recorded. The lungs were ventilated at constant tidal volume by a positive pressure pump. Tidal CO_2 was monitored continuously, and maintained within normal limits. We measured systemic and pulmonary arterial blood pressure and tracheal pressure. Drugs were injected into the right and left atrium, or administered as aerosols generated by an ultrasonic nebuliser (DeViblis). Some afferent vagal C-fibre endings in the lung are located in structures supplied by the pulmonary circulation, others are in structures supplied by the bronchial circulation¹¹. However, PGs had similar effects on these two groups of C-fibres, and the results have been combined.

Injection of $\text{PGF}_{2\alpha}$ ($1-8 \mu\text{g kg}^{-1}$) into the right atrium stimulated all 23 'irritant' receptors examined. These doses are within the range used by Wasserman⁵ to produce changes in airway mechanics in anaesthetised dogs. In the majority of experiments reported here we did not examine the dose-response relationship in detail because the recording life of the fine vagal strands was often limited; and we had to determine the precise location of the ending before the fibre became inactive. In most cases, therefore, we used a dose of $4 \mu\text{g kg}^{-1}$ to examine the response of the ending. Latencies of onset ranged from 5 to 25 s (mean 12.2). Activity increased from

1.7 ± 0.4 impulses s^{-1} (mean \pm s.e.) during the control period to 10.4 ± 2.0 impulses s^{-1} , when averaged over one respiratory cycle at the peak of the response. Firing remained above control for 30-120 s (mean 73.5). There was invariably an increase in peak tracheal pressure ($2.1 \pm 0.3 \text{ cm H}_2\text{O}$). The observed cardiovascular effects, consisting of an increase in pulmonary arterial pressure, and usually a small rise in systemic arterial pressure, confirm earlier findings¹². 'Irritant' receptor firing and tracheal pressure also increased when an aqueous solution of $\text{PGF}_{2\alpha}$ ($0.05-0.1 \text{ mg ml}^{-1}$) was administered as an aerosol. A typical response to $\text{PGF}_{2\alpha}$ injected into the right atrium is seen in Fig. 1.

The majority of 'irritant' receptors are believed to be in the larger airways¹³, which are supplied by the bronchial circulation. Nevertheless, injection of $\text{PGF}_{2\alpha}$ ($2-12 \mu\text{g kg}^{-1}$) into the left atrium stimulated only one out of eight receptors tested; tracheal pressure increased on average by only $0.2 \text{ cm H}_2\text{O}$, and arterial pressures were unchanged. Thus $\text{PGF}_{2\alpha}$ stimulates 'irritant' receptors regularly only when it has access to the pulmonary circulation, from which site it produces its maximal bronchoconstrictor effects. This observation, coupled with evidence that PGs are largely destroyed in a single passage through the lung¹, favours the hypothesis that 'irritant' receptor stimulation is not primarily due to the effects of $\text{PGF}_{2\alpha}$ on the nerve ending, but is secondary to changes in lung mechanics. This interpretation was strengthened by our finding that administration of an aerosol of isoproterenol ($0.1-0.2 \text{ mg ml}^{-1}$) significantly reduced the effect of $\text{PGF}_{2\alpha}$ on both receptor activity and tracheal pressure (Table 1). Similarly, when PGE_2 ($10-20 \mu\text{g kg}^{-1}$), which has been shown to reduce the bronchoconstrictor effect of $\text{PGF}_{2\alpha}$ ^{14,15}, was injected into the right atrium 1-6 min before injection of $\text{PGF}_{2\alpha}$, there was a significant reduction in the effect of $\text{PGF}_{2\alpha}$ on both receptor firing and tracheal pressure. However, in experiments in which we used isoproterenol, PGE_2 , or atropine to antagonise the bronchoconstrictor effects of $\text{PGF}_{2\alpha}$, there were a few instances in which 'irritant' receptor stimulation by $\text{PGF}_{2\alpha}$ was unchanged. Hence the possibility that $\text{PGF}_{2\alpha}$ also stimulates 'irritant' receptors directly cannot be ruled out.

If $\text{PGF}_{2\alpha}$ has a causative role in asthma⁴, then our finding that $\text{PGF}_{2\alpha}$ stimulates 'irritant' receptors acquires considerable pathophysiological significance. It has been postulated that stimulation of 'irritant' receptors by histamine causes a reflex bronchoconstriction which is superimposed on the direct action of the drug^{16,17}. The same may be true for $\text{PGF}_{2\alpha}$.

There was a similar but less pronounced stimulation of 14 of 19 lung C-fibres when $\text{PGF}_{2\alpha}$ ($2-12 \mu\text{g kg}^{-1}$) was injected into the right atrium, impulse frequency for the group as a whole

Table 1 Reduction of effects of $\text{PGF}_{2\alpha}$ by isoproterenol aerosol

		'Irritant' receptor activity (impulses s^{-1})	Peak tracheal pressure (cm H_2O)
A	Control	1.4 ± 0.5	10.9 ± 0.5
	$\text{PGF}_{2\alpha}$	12.6 ± 2.1	12.9 ± 0.7
B	Control	2.1 ± 0.6	10.9 ± 0.5
	$\text{PGF}_{2\alpha}$	4.5 ± 0.7	11.2 ± 0.5

Effects on 'irritant' receptor activity and tracheal pressure of injecting $\text{PGF}_{2\alpha}$ ($2-8 \mu\text{g kg}^{-1}$) into the right atrium. Results comprise 13 complete trials on eight receptors. The data in the upper two lines (labelled A) were obtained before administration of isoproterenol; data in the lower two lines (labelled B) were obtained after an aerosol of isoproterenol ($0.1-0.2 \text{ mg ml}^{-1}$ solution) had been administered for 5 min. Mean values are given together with their standard errors. The reduction by isoproterenol of the effects of $\text{PGF}_{2\alpha}$ was highly significant ($P < 0.002$).

increasing from 0.5 ± 0.1 to 2.1 ± 0.5 impulses s^{-1} (Fig. 1). The latency of response was 24 ± 2.5 s, and firing remained above control levels for 123 ± 20.8 s.

Surprisingly, PGs of the E series, which are known to be highly irritant^{6,7}, had only minor effects on 'irritant' receptors, only 6 of 12 receptors being stimulated by PGE_2 ($10\text{--}20 \mu g \text{ kg}^{-1}$) injected into the right atrium. The increase in activity for the group as a whole was only 1.4 ± 0.5 impulses s^{-1} and the effect lasted no more than four to five respiratory cycles. On average, peak tracheal pressure decreased by $0.1 \text{ cm H}_2\text{O}$. PGE_2 ($5\text{--}10 \mu g \text{ kg}^{-1}$ injected into the right atrium), however, was a powerful stimulant of 19 of 21 lung C-fibre endings, impulse activity increasing from 0.6 ± 0.1 to 5.9 ± 0.9 impulses s^{-1} (with a latency of response of 14.7 ± 0.8 s) (Fig. 1). The effect, moreover, was extremely prolonged (average duration 6.5 min); indeed 15 min sometimes elapsed before firing returned to control levels. This was surprising in view of the reported rapid inactivation of PGs¹. The concomitant hypotensive effect also was long-lasting, a finding that agrees with earlier observations on PGE_1 ¹². We have no explanation for these prolonged effects.

In the same dose range, PGE_1 had similar but less pronounced effects on C-fibre endings. The relative potencies of PGE_1 and PGE_2 on C-fibre endings parallel the observation that, in man, the irritant properties of PGE_1 are less than those of PGE_2 (ref. 7). Furthermore, in our experiments, administration of isoproterenol aerosol did not reduce the stimulation of lung C-fibre endings by PGE_2 , and in man isoproterenol does not prevent the cough response to PGE_2 (ref. 6). Hence it is conceivable that C-fibres, rather than 'irritant' receptors, are responsible for PGE -induced cough.

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Multiple sclerosis, Guillain-Barré syndrome and myelin basic protein-specific cellular antibody

MULTIPLE sclerosis (MS) and the Guillain-Barré syndrome (GBS) are demyelinating diseases of unproven aetiology and

pathogenesis, in which infectious and immunological factors have been suggested to have a role^{1–3}. The notion that sensitisation to basic proteins from myelin might be involved has arisen from certain similarities between these human diseases and the autoimmune demyelinating diseases of animals, experimental allergic encephalomyelitis (EAE) and experimental allergic neuritis (EAN)⁴. EAE is caused by sensitisation to the encephalitogenic basic protein from central myelin (CNS-BP) and EAN by sensitisation to peripheral myelin. One of the two basic proteins of peripheral myelin, P2 protein⁵, is considered a likely candidate for the neuritogenic factor⁶. To help determine whether sensitisation to myelin basic proteins is involved in the pathogenesis of MS and GBS, we have used horseradish peroxidase (HRP)-labelled myelin basic proteins^{7,8} to seek evidence of an antibody of this specificity in nervous system plasma cells in these diseases. Our negative findings do not support a pathogenetic role for myelin basic proteins in MS or GBS.

In rabbits with EAE, it has been shown that plasma cells specifically binding a conjugate of CNS-BP and HRP are present in tissue sections of lymph nodes from day 8 until at least day 54 after immunisation^{9,10}. Such cells are also present in early spinal cord lesions¹¹, the only time at which they have been sought. Likewise in rabbits with acute EAN, plasma cells specifically binding an HRP conjugate of P2 protein are present in lymph nodes and dorsal root ganglia (DRG)⁴. The binding of these conjugates by plasma cells in these disorders is believed to reflect the presence of intracellular IgG antibody specific for the respective basic proteins¹⁰. The conjugation procedure diminishes somewhat the immunological reactivity of CNS-BP (refs 11, 12), the method does not detect plasmalemmal staining as might be anticipated to occur on CNS-BP reactive lymphocytes (our unpublished observations), and no relationship has been proved between the reactive plasma cells and the pathogenesis of EAE or EAN. Nevertheless, the presence of these basic protein-binding cells serves as an indicator of the presence of immunological reactivity towards the particular antigen, and their presence in the nervous system lesions puts them in a position of potential pathogenetic importance.

There is no conclusive evidence in MS and GBS that sensitisation to myelin basic proteins is involved in pathogenesis. A relationship was, however, recently reported between acute exacerbations of MS and sensitisation to CNS-BP, as measured by a macrophage migration inhibition assay¹³, and sensitisation to peripheral nerve basic proteins has been noted in GBS (refs 14, 15). Whether these sensitisations are primary or secondary features of the diseases is not known. Partly because signs of EAE can be suppressed by injections of CNS-BP and some other compounds¹⁶, trial treatment of MS with CNS-BP or other EAE-suppressive compound is being considered. In MS, IgG antibody is believed to be produced locally within the nervous system¹⁷. MS cerebrospinal fluid contains increased levels of oligoclonal immunoglobulins¹⁸ and increased numbers of IgG-containing cells¹⁹, both of unproven antibody specificity. If plasma cells specifically binding myelin basic proteins were present in MS or GBS, this would support the suggestion that sensitisation to myelin basic proteins may have a role in these diseases.

We have sought cells binding an HRP conjugate of CNS-BP in fresh frozen tissue obtained at autopsy from three chronic cases of MS. Formalin-fixed cryostat sections of nine cerebral MS plaques with adjacent white matter were stained with oil red O and methyl green-pyronin as well as with a light microscopic, peroxidase-CNS-BP conjugate procedure described previously⁷. All the plaques exhibited disease activity, as indicated by the presence of considerable numbers of fat-laden macrophages at the periphery, and four were quite active with lipid macrophages throughout.

Perivascular mononuclear infiltrates and scattered pyronophilic plasma cells, both within the cuffs and free in the parenchyma, were present in seven plaques. Two additional blocks of normal appearing white matter containing scattered pyronophilic mononuclear cells were also studied, as was one lymph node from the case with three of the most active plaques. In addition, we assayed smears of cerebrospinal fluid sediments containing mononuclear cells from four other MS patients and two controls. All these tissues and cells were incubated either with a conjugate of bovine or human CNS-BP, or both. Although these two conjugates both gave positive results in lymph nodes of rabbits immunised with bovine CNS-BP, in none of the MS tissues or cells was specific binding of CNS-BP demonstrable.

Fresh frozen tissue from two fatal cases of GBS was similarly tested, but in this instance for binding of an HRP conjugate of bovine P2 protein⁸. Formalin-fixed cryostat sections of five lumbar and three sacral DRG and two peripheral nerves generally contained mononuclear infiltrates and rare plasma cells, but no specific binding of P2 protein was found. The P2 protein conjugate did reveal positive plasma cells when used with lymph nodes and DRG from rabbits immunised with bovine peripheral myelin.

In contrast to the situation in EAE and EAN, these studies do not support the existence of sensitisation to basic proteins from myelin in either MS or GBS, as measured by cellular anti-basic protein antibody. Serum and cerebrospinal fluid anti-basic protein antibodies also are reported not to be detectable in MS^{20,21}. Since in both EAE and EAN, only a portion of the plasma cells bind basic protein conjugates, sampling of the human material could constitute a limitation of this study. Little is known about the species cross reactivity of P2 protein, but species differences are unlikely to be a factor in the MS results of this study. Both human and bovine CNS-BP were tested, and wide species cross reactivity between human, bovine, rabbit, rat and guinea pig CNS-BP has been demonstrated with this peroxidase procedure (A.B.J., unpublished observations) and by another technique²².

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Proposed respiratory 'gating' mechanism for cardiac slowing

THREE of the essential variables concerned in the regulation of systemic cardiovascular performance are cardiac output, blood pressure and the resistance encountered by the blood in flowing through the systemic vascular bed. Cardiac output may be further considered as the product of cardiac stroke volume and heart rate. The latter is under the control of the vagi and sympathetic nerves but the reflex bradycardia produced by vagal activation as a result of increased blood pressure, increased carotid chemoreceptor activity or direct electrical stimulation of the carotid sinus nerve occurs only if the stimulus is delivered during expiration. A sustained stimulus

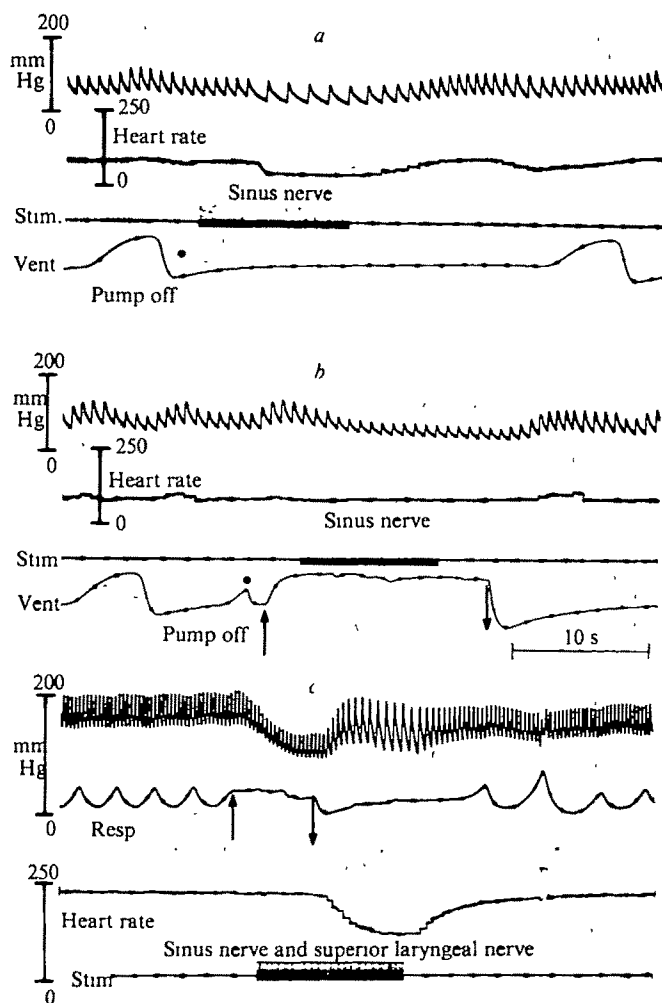


Fig. 1 *a* and *b*, Spinal cord cut at level of first cervical vertebra; cat ventilated with respiratory pump. *a*, Pump switched off and sinus nerve stimulated producing bradycardia; *b*, pump switched off and sinus nerve subsequently stimulated during period of sustained lung inflation (between arrows); no bradycardia. *c*, Intact animal; natural breathing. Sustained lung inflation between arrows. Combined sinus nerve and superior laryngeal nerve stimulation begun during this inflation; only effective in producing bradycardia on deflation of lungs.

which is able reflexly to activate the cardiac vagal efferents will, therefore, produce the pattern of heart rate known as sinus arrhythmia (that is, a slow heart rate regularly interrupted by periods of cardiac acceleration coinciding with inspiration). This association of inspiratory activity with the inhibition of bradycardia is also seen when stimulating certain areas of the brain: areas which produce an inhibition of bradycardia^{2,3}

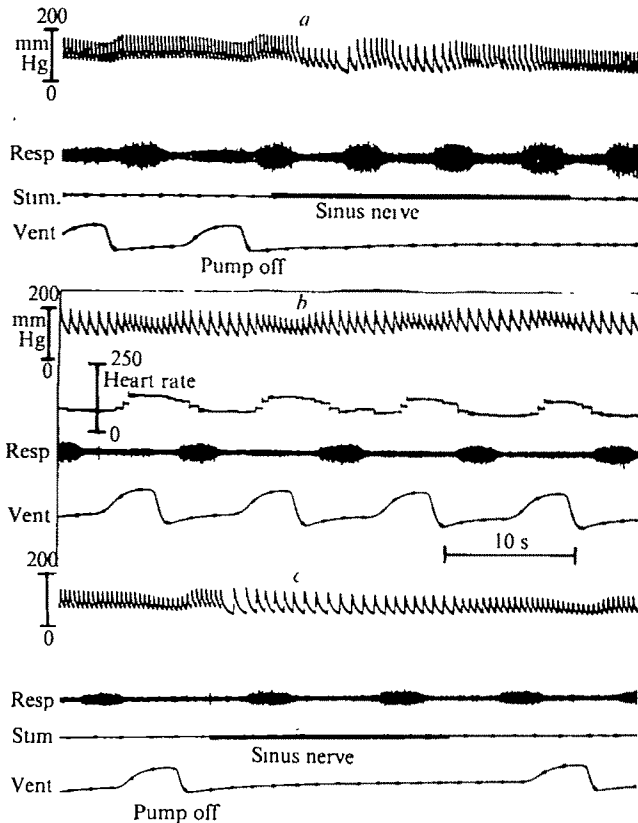


Fig. 2 Spinal cord sectioned at level of first cervical vertebra; cat ventilated with respiratory pump. Central inspiratory drive indicated by recurrent laryngeal electroneurogram. In *a* the pump is switched off and the sinus nerve stimulated at a strength that increases the intensity and frequency of the central inspiratory drive, which is clearly in phase with the periods of cardiac acceleration of the sinus arrhythmia. (The R_a neurones are then driving the R_b neurones?) In *c* the sinus nerve is stimulated at a reduced strength following a long period of artificial ventilation, and a bradycardia with much less sinus arrhythmia is seen. (The R_b neurones are perhaps less active under these conditions.) In *b* when the lungs are artificially ventilated with an increased stroke volume the periods of cardiac acceleration now correspond with lung inflation and not with the central inspiratory drive. (Possibly in these conditions the R_b neurones, which are powerfully provoked by artificial lung inflation, are insensitive to the natural R_a discharge.)

also produce an increase in respiratory activity, while other areas produce bradycardia and apnoea⁴. A vagal bradycardia unrelated to respiration can in fact only be induced by stimulating the cells of origin in the nucleus ambiguus^{5,6} or the vagus nerve itself. This nucleus lies in the medullary reticular formation ventro-lateral to the nucleus of the tractus solitarius. Since all known afferent stimuli which cause reflex bradycardia converge on the area of the nucleus tractus solitarius where "inspiratory" type neurones abound before passing on to the nucleus ambiguus, it is tempting to postulate that these inspiratory neurones may actually be part of a 'gate' which controls the passage of impulses through this reflex pathway. The rhythmical opening and closing of this gate with respiratory activity would result in sinus arrhythmia provided the gate remains open for a sufficient length of time and that there is an adequate background of vagal tone, which is in itself reflexly engendered. We have experimentally analysed some of the

properties of this proposed respiratory gating mechanism and conclude that it is in fact influenced by both neural inspiratory activity and by lung inflation.

Normally the central inspiratory drive and the resulting lung inflation are almost in phase with the inhibition of cardiac vagal tone. Both the central inspiratory drive and the resulting lung inflation may, however, be abolished by stimulation of the superior laryngeal nerve⁷ which inhibits the neural discharge to both inspiratory and expiratory muscles. If an adequate background level of afferent activity exists, such as is provided by baroreceptor discharge, bradycardia will occur as there is now no hindrance to the afferent impulse traffic to the nucleus ambiguus.

When both aortic and sinus nerves are cut, no bradycardia occurs on stimulation of the superior laryngeal nerve. This shows that stimulation of this nerve does not in itself induce bradycardia. If, in this preparation, the central end of a carotid sinus nerve is stimulated together with the superior laryngeal nerve, profound bradycardia occurs.

Although the superior laryngeal nerve is one of the most powerful means of opening the gate it is not in itself the "master key". If the lungs are kept artificially inflated, not even simultaneous stimulation of the sinus and superior laryngeal nerves produces bradycardia (Fig. 1c). As soon as the lungs are deflated this stimulation now provokes bradycardia.

After spinal transection at the level of C1, and with the lungs artificially ventilated, the vagal afferent traffic virtually becomes the only source of information concerning lung volume; in this situation, stimulation of the sinus nerve only elicits bradycardia when artificial respiration is discontinued with the lungs deflated (Fig. 1a and b). Thus inflation receptors may have a role in the genesis of sinus arrhythmia. It may be seen in Fig. 1a that, with the pump switched off, the heart rate is still being modulated by the central respiratory drive, and thus the onset of bradycardia provoked by sinus nerve stimulation is delayed by over 4 s presumably until the central inspiratory drive has temporarily ceased.

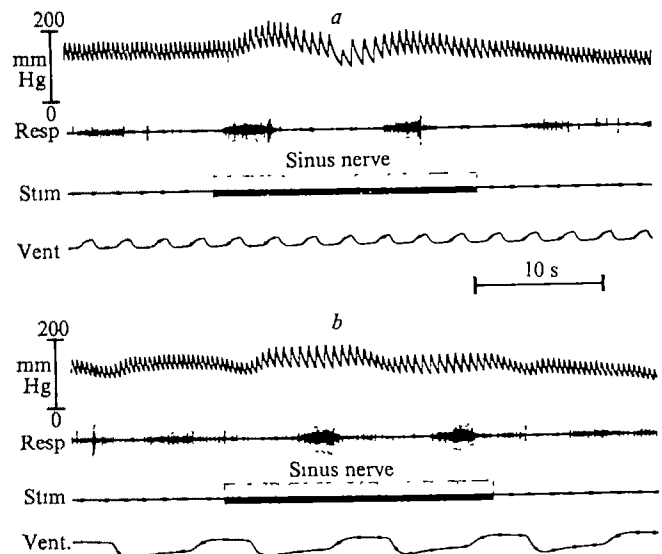


Fig. 3 Intact animal with open chest; ventilation of lungs with pump. Intercostal CMG to indicate central inspiratory drive. *a*, Pump stroke volume 75 ml; sinus nerve stimulation provokes sinus arrhythmia in which periods of cardiac acceleration correspond with central inspiratory drive and are not in phase with the pump. (R_b are being driven by R_a neurones and are insensitive to small volume high frequency ventilation.) *b*, Pump stroke volume 150 ml; periods of cardiac acceleration in sinus arrhythmia now correspond with lung inflation and are not in phase with central inspiratory drive. (R_b are driven by high volume low frequency lung inflation and are insensitive to natural discharge of R_a neurones.)

It is however also possible to show that in such spinal animals the central respiratory drive can by itself operate this 'gate' in the absence of lung inflation. In Fig. 2a artificial ventilation is temporarily discontinued during which time the sinus nerve is stimulated. In this circumstance sinus arrhythmia can still be observed with its periodic inhibition of bradycardia now in phase with the increased central respiratory drive. When artificial respiration is resumed, with an increased stroke volume bradycardia becomes associated with lung deflation rather than with the expiratory phase of the central respiratory rhythm (Fig. 2b).

Another method of dissociating the dual influence of central drive and lung inflation is to thoracotomise an otherwise intact anaesthetised animal and to ventilate its lungs with a pump. When the respiratory minute volume is adjusted to maintain normal values of pO_2 and pH at low tidal volume and high frequency, the central respiratory rhythm (recorded electromyographically from intercostal muscles) is slower than the prevailing pump frequency: sinus arrhythmia produced by sinus nerve stimulation now occurs in phase with this central rhythm (Fig. 3a). When tidal volume is increased and the rate of pumping slowed, so as to still preserve normal blood gas tensions and pH , the pump drives the central rhythm "out of phase", and now lung deflation occurs during central inspiratory drive: sinus arrhythmia produced by sinus nerve stimulation is now locked to the lung volume changes, with bradycardia in phase with lung deflation (Fig. 3b).

These results suggest the following propositions. (1) The gating mechanism, which may be complex, is probably located in association with both the nucleus tractus solitarius and with the nucleus ambiguus. (2) Complete inhibition of central respiratory drive, such as induced by stimulation of the superior laryngeal nerve, fully opens this 'gate' if, and only if, the lungs are in a state of deflation. (3) The gating mechanism can be operated independently by central drive or by lung inflation. (4) When the lungs are markedly inflated the pulmonary vagal afferent discharge is prepotent in closing the gate.

These considerations strongly suggest that a group of neurones first described by Baumgarten and Kanzow⁸ may qualify as an integral part of this gating mechanism. They described two types of inspiratory neurones in the vicinity of the nucleus tractus solitarius and named them R_a and R_b . The R_a neurones are centrally driven and discharge in phase with the phrenic motor neurones even when the respiratory muscles are paralysed with succinyl choline and both vagi are divided; these R_a neurones are inhibited by lung inflation. The R_b neurones discharge in phase with the R_a , even after succinyl choline and bilateral vagotomy, but unlike the R_a or the phrenic neurones, are stimulated by lung inflation.

R_a neurones do not fulfil the requirements necessary to operate as a gating mechanism as they do not discharge during lung inflation when the gate is shut. R_b neurones, on the other hand, do possess the necessary characteristics. Situated as they are, close to the nucleus tractus solitarius, they could operate in conjunction with the afferent stimulus, for it is known that the "gate" can function within one or two synapses of the afferent inflow⁶. They are independently or simultaneously driven both by central respiratory drive and by lung inflation and are correspondingly active in both situations of "gate" closure. It is possible that their extra discharge induced by powerful lung inflation leads to their becoming temporarily insensitive or 'refractory' to the central command. Such a characteristic might explain the occurrence, under special conditions, of bradycardia coincidental with central inspiratory drive but blocked by lung inflation (Figs 2b, 3b). Similarly after a period of powerful ventilation it is also possible, on suspending artificial ventilation and stimulating the sinus nerve, to induce bradycardia uninterrupted by the tachycardia normally associated with central inspiratory drive (Fig. 2c). Lipski, McAllen and Spyer¹⁰ have analysed the response of R_b neurones to chemoreceptor stimulation in artificially ventilated anaesthetised cats and while finding that the R_b neurones could

be driven by the chemoreceptors during inspiration (with closure of the proposed 'gate'), they also found that the discharge of the R_b neurones during sustained lung inflation was diminished by chemoreceptor stimulation. This might be due to the previously mentioned insensitivity of the R_b neurones to the central inspiratory drive associated with a period of sustained lung inflation and the unmasking of an inhibitory influence of the chemoreceptors on the R_b neurones. Such an influence would be clearly beneficial in the chemoreceptor-induced bradycardia that occurs when efferent respiratory activity is also suspended by simultaneous stimulation of the superior laryngeal nerve¹¹. The presence of ungated baroreceptor units in the vicinity of the nucleus ambiguus in no way negates the 'gating' hypothesis as the final inhibitory neurone of the gate mechanism may terminate directly on the cell bodies of the nucleus ambiguus.

The R_b neurones, therefore, appear as strong candidates for incorporation in the neuronal organisation of this proposed respiratory 'gate'.

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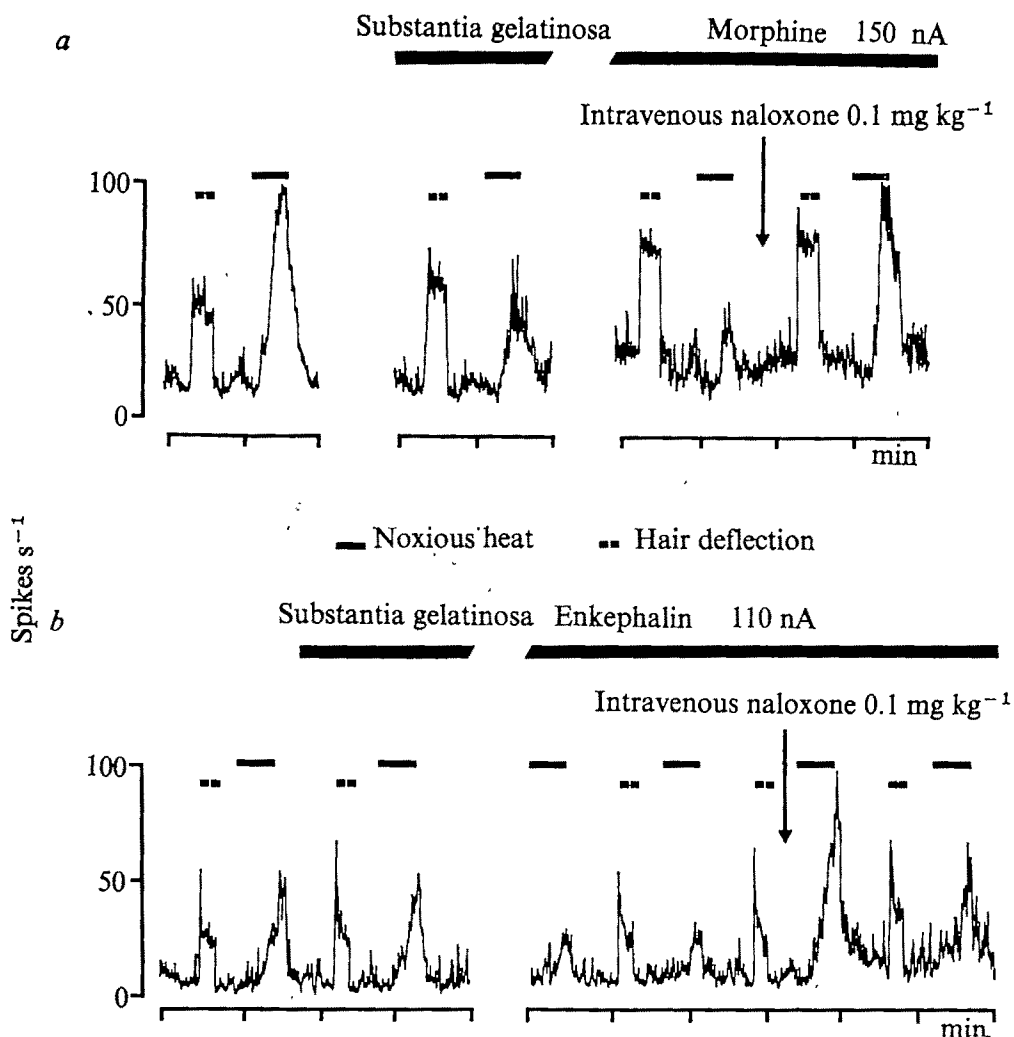
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Morphine, enkephalin and the substantia gelatinosa

THE pentapeptide enkephalin interacts with opiate receptors of peripheral tissues¹ and with stereospecific opiate receptors of brain homogenates². Ejected from micropipettes in the vicinity of central neurones, it has depressant³⁻⁵ and excitant⁶ effects. These actions, however, cannot be related specifically to the analgesic effects of systematically administered opiates. In electrophoretic experiments, tissue concentrations are unknown and hence even antagonism by electrophoretic naloxone is not necessarily a sufficient test of relevance to the effects of systemic opiates. This report is concerned with the effects of morphine when administered electrophoretically into the substantia gelatinosa. The subsequent changes in the firing of neurones within Rexed laminae IV and V can be related to the effects of this compound given systemically. Furthermore methionine enkephalin had an action similar to that of morphine.

Experiments were performed on cats anaesthetised with α -chloralose. The spinal cord was divided at the thoracolumbar junction. In preliminary experiments morphine (0.07 M) and naloxone (0.1 M, Endo Laboratories) were ejected electrophoretically from the outer barrels of 7-barrel micropipettes and the centre barrels were used for extracellular recording. Drug administration was thus largely into the region of cell bodies. Neurones of spinal laminae IV and V were excited alternately by radiant heat adequate to raise the skin temperature of a hind limb digital pad above 45 °C (a noxious stimulus), and

Fig. 1 The effect of morphine (*a*) and methionine enkephalin amide (*b*) on the firing of lamina IV spinal neurones by a noxious and non-noxious skin stimulus and the reversal of these effects by intravenous naloxone (0.1 mg kg^{-1}). In both *a* and *b* the noxious stimulus was heat to the fourth digital pad of the left hind leg adequate to raise skin temperature above 45°C ; the non-noxious stimulus was deflection of adjacent hairs by a moving air jet. In *a*, 14 min elapsed between the recording of the left and middle groups of responses and 55 min between the middle and right groups. In the latter interval, naloxone, ejected electrophoretically into the substantia gelatinosa, was shown to antagonise the effects of morphine. In *b*, 12 min elapsed between the right and left groups of records.



by deflection of the adjacent hairs by a moving air jet (a non-noxious stimulus). In these conditions morphine failed to reduce selectively the activation of cells by either stimulus, and high ejecting current (80–100 nA) produced abnormalities in action potential amplitude and configuration⁸.

In marked contrast, when morphine was ejected from another micropipette located superficial to the cell body and positioned in the region of the substantia gelatinosa, selective depression of cell responses to the noxious stimulus was observed without effect on those to the non-noxious stimulus (11 to 14 cells). The separation between the tips of the drug-administering and the recording micropipettes varied from 100 to 830 μm . Results from one neurone are illustrated in Fig. 1*a*. The middle records show that after 14 min of administration of morphine with an ejecting current of 150 nA, there was approximately a 50% reduction in the response to painful stimuli while the non-pain response was slightly increased. The reduction in the response to painful stimuli by morphine was long lasting, commonly greater than 30 min. This effect of morphine was reversed by naloxone administered electrophoretically in the substantia gelatinosa, and, more significantly, by intravenous naloxone in doses as low as 0.1 mg kg^{-1} . This dose of naloxone reverses the effects of analgesic doses of morphine in the cat⁹ and dog¹⁰ and its effectiveness in our experiments strongly suggests that the concentrations of morphine in the substantia gelatinosa reducing responses to painful stimuli were not inappropriate to those present after systemic morphine. The rapid effects of intravenous naloxone are shown in Fig. 1*a*.

Two samples of enkephalin were ejected from pipettes positioned in the substantia gelatinosa; methionine enkephalin (Calbiochem, 0.015 M, pH 3.5) and methionine enkephalin amide (supplied by Drs H. Niall and G. Tregear, The Howard Florey Institute, Melbourne; 0.020 M, pH 4). Of 17 cells tested, enkephalin ejected into the substantia gelatinosa reduced the responses of 12 to painful stimuli. With six neurones this reduction occurred in the absence of any effect on responses to painless stimulation or on spontaneous firing, with five the predominant effect was a reduction in the response to painful stimuli with a smaller reduction in the response to painless ones, and with one cell all three firing patterns were reduced in parallel. In the latter case the separation between the electrode tips was the smallest used. More consistent effects were observed with methionine enkephalin amide (10 of 11 cells tested) than with methionine enkephalin (2 of 6 cells). Figure 1*b* shows the selective reduction of the responses to painful stimulation of a lamina IV neurone after 13 min of ejection of methionine enkephalin amide (110 nA) in the substantia gelatinosa. In contrast to morphine, recovery from the effects of enkephalin was rapid, being complete within 10 min of the termination of ejection.

The reduction of responses to painful stimulation by enkephalin was fully reversed in 5 of 6 animals by a low dose of intravenous naloxone (0.1 mg kg^{-1} in four cats, and 0.3 mg kg^{-1} in one). The effect of naloxone was rapid as illustrated in Fig. 1*b*. All observed effects of enkephalin were reversed by naloxone—when response to non-painful stimulation and spontaneous firing were depressed by enkephalin this depression was also reversed by intravenous

naloxone. With one neurone, intravenous naloxone 0.1 mg kg^{-1} partially reversed the effects of enkephalin; this effect was not increased by additional doses up to a total of 1.2 mg kg^{-1} .

These results suggest that a receptor for morphine in the substantia gelatinosa is indeed relevant to the reduction, by systemic opiates, of pain with minimal effect on other sensations¹¹. The substantia gelatinosa contains the endings of primary afferent fibres, dendrites of deeper neurones, and small neurones which project only to other parts of the substantia gelatinosa. Axo-axonic synapses are abundant¹². Autoradiographic¹³ and binding studies¹⁴ have shown this to be a region of high opiate binding, but further experiments are needed to determine the fine structural localisation of the morphine receptors of the substantia gelatinosa and their relevance to primary afferent fibres and interneurons associated with painful stimuli. The methods used in the present experiments offer a means for determining the possible physiological role of enkephalin.

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Intraventricular Met⁵-enkephalin causes unexpected lowering of pain threshold and narcotic withdrawal signs in rats

PEPTIDES with opiate properties have been demonstrated in brain^{1–3} and pituitary^{4–7}. Goldstein⁸ has postulated that sustained low-intensity pain might promote the central mobilisation of endogenous opioid as part of an adaptive response to noxious stimuli which cause suffering but do not threaten survival. We have examined the effects of intraventricular infusion of an endogenous opioid peptide, methionine-enkephalin⁹ (Met⁵-enkephalin), on responses to a sustained mildly noxious stimulus⁹ in rats. We expected Met⁵-enkephalin to attenuate responsiveness to the noxious stimulus, as has been reported¹⁰ for its effects on acute pain. On the contrary in our experiments, however, it seems to have increased responsiveness, and moreover to have induced behaviour typical of opiate withdrawal.

Thirteen male Sprague-Dawley albino rats, 180–200 g, were implanted under pentobarbitone anaesthesia (Nembutal, 50 mg kg⁻¹) stereotactically with chronic intraventricular cannulae in the right lateral ventricle (De Groot¹¹: A-3.2, L-5.0, V-5.1) according to the technique of Rezek and Havlicek¹². Correct placement of cannulae and success of infusion were confirmed histologically. Rats were first tested (test 1) 8 d after cannula implantation. They were given intraventricular infusions of either 10 μl saline over 65 s (saline control group of test 1) or of 100 μg Met⁵-enkephalin (Beckman Bioproducts) in an identical volume and rate of saline infusion. At 1.0 min after the end of

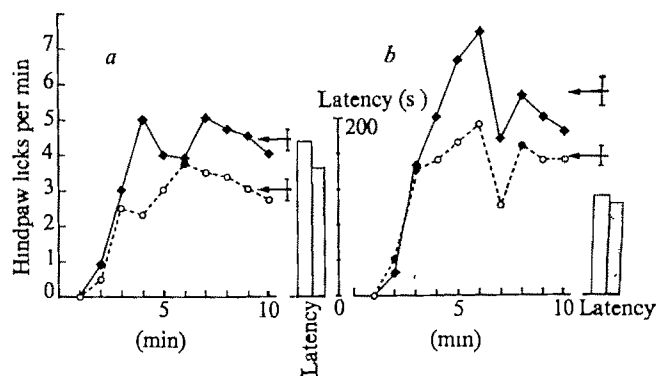


Fig. 1 *a*, Effect of Met⁵-enkephalin (stippled column and \blacklozenge) via right lateral ventricular infusion on hindpaw licks in rats kept on 44.5 °C hotplate for 10 min (test 1 of cross-over test). For a given group, each point on graphs indicates average number of hindpaw licks per animal observed over the minute preceding the plotted time. Height of arrowed horizontal lines above abscissa indicates hindpaw licks per min over last 7 min of the 10-min test; their vertical lines show standard errors. Six rats were infused with Met⁵-enkephalin, seven with saline (open column and \circ) as controls. Latency is time in s when first hindpaw lick was observed after rat was placed on hotplate; height of latency bar represents mean for each group. *b*, Test 2 of crossover test. Rats in Met⁵-enkephalin group ($n = 5$) were all taken from those in the saline group of test 1; rats in the saline group ($n = 4$) were all taken from those in Met⁵-enkephalin group of test 1. Other details as in *a*.

infusion the animals were placed within a vented transparent cylinder (48 cm high \times 20 cm in diameter with top open) on a hotplate at 44.5 °C (ref. 9). There was a significantly ($P \leq 0.01$) higher frequency of hindpaw licking in the Met⁵-enkephalin-infused group than in the saline controls, evaluated over the last 7 min of the 10-min test (Fig. 1*a*). Significant differences did not occur between hindpaw licking frequency in the two groups over the first 3 min, nor between latencies to the first hindpaw lick (Fig. 1*a*).

All animals with cannulae still intact after 5 d were retested (test 2) in crossover fashion; those which had received saline in test 1 were given infusions of Met⁵-enkephalin, and vice versa. Again, the Met⁵-enkephalin-infused group exhibited significantly ($P \leq 0.02$) more hindpaw licking than did the saline-infused rats (Fig. 1*b*), thus confirming results of the first test. Mean latency to first hindpaw lick was shorter in the Met⁵-enkephalin-infused rats in test 2 (Fig. 1*b*) than in the same animals during infusion with saline in test 1 (Fig. 1*a*). The difference was significant ($P \leq 0.05$) by single-tailed *t*-test for paired comparisons.

In both tests, all the animals infused with Met⁵-enkephalin exhibited signs of narcotic withdrawal¹³, that is: wet dog shakes, rearing, sniffing, restless exploring activity and increased defaecation, beginning at 1–3 min after the start of infusion. In some animals these signs were particularly severe. None of the rats showed these signs when infused with saline in either test.

Six of the rats used in tests 1 and 2 were retested on the hotplate, 7 d after test 2, with infusion of 2.0 μg naloxone hydrochloride (Endo Laboratories) in 10 μl saline/65 s. The animals showed significantly ($P \leq 0.05$) more hindpaw licking than did the saline-infused animals of either test 1 or 2 but displayed no signs of narcotic withdrawal. Retesting three of these animals with 15.0 μg naloxone 10 d later gave a similar result.

The hotplate test used here differs from the conventional hotplate test for analgesia in that the surface temperature is low enough to permit exposure of the experimental animal to a 10-min sustained mildly noxious environment in a demarcated space. It therefore permits observations to be made on the effects of drugs on the behavioural responses to such exposure; hindpaw licking is a prominent feature of those responses in rats. We believe it justified to regard hindpaw licking as an indication of pain, since it is readily distinguishable from

normal grooming activity and is much reduced by morphine given either intraperitoneally⁹ or intraventricularly. The effects of intraventricular Met⁵-enkephalin in causing an apparent increase in perception of pain, as well as signs of opiate narcotic withdrawal, were totally unexpected.

Leucine-enkephalin (Leu⁵-enkephalin) (Peninsula Laboratories) was tested in a separate series of experiments with 18 rats, also chronically implanted with intraventricular cannulae. Three groups of six rats were first tested after infusion of saline. In a second test 6 animals were given saline, morphine sulphate (10 µg), or Leu⁵-enkephalin (100 µg) (Fig. 2). It can be seen that morphine and Leu⁵-enkephalin significantly diminished hindpaw licking in response to the hotplate. Thus, Leu⁵-enkephalin, in contrast to Met⁵-enkephalin, exhibits analgesic action like that of morphine.

Our findings with Met⁵-enkephalin may be explained by three main factors: (1) inability of the pentapeptide opioid to reach sites of opiate analgesic action in caudal portions of the ventricular system¹⁴; such failure could be due to rapid destruction of Met⁵-enkephalin by brain peptidases¹⁵ and, perhaps, to poor lipid solubility; (2) excitation, by Met⁵-enkephalin, of sites of "paradoxical" opiate action^{16,17}; (3) the conditions of our hotplate test, which may induce the mobilisation and release of endogenous opiate-like substances in the brain, their target being stereospecific opiate receptor sites concerned with the attenuation of pain perception.

It is conceivable that among the released endogenous opioids there exists an unidentified opioid substance which is a more potent opiate agonist than Met⁵-enkephalin. After several minutes of stimulation by such an agonist, the system may adapt to, and thus become "dependent" on, the potent opioid; very rapid acquisition of opiate narcotic tolerance and physical dependence has been seen in several studies¹⁸⁻²⁰. The intraventricular infusion of Met⁵-enkephalin in such an opioid-

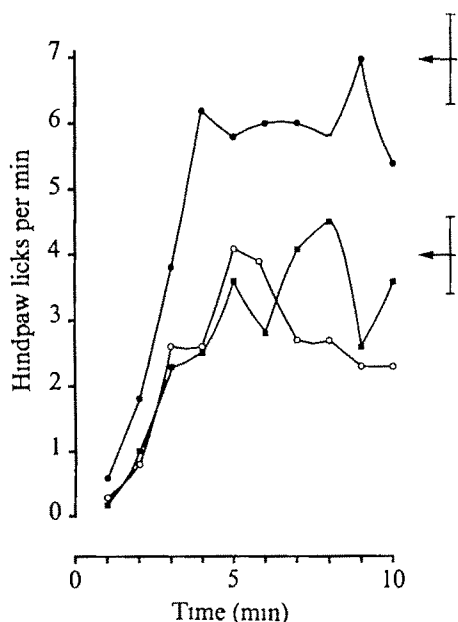


Fig. 2 Effect of Leu⁵-enkephalin (■), morphine (○) or saline (●) on rats subjected to a hotplate. Other details as in Fig. 1.

adapted brain would result in the displacement of the more potent endogenous opiate-like substance, with the consequent signs of narcotic withdrawal and hypernociception that we observed in our experiments. Studies³ on isolated organs *in vitro* have shown that Met⁵-enkephalin has predominantly opiate agonist properties. Nevertheless, its sodium sensitivity in opiate receptor binding studies⁷ suggests that Met⁵-enkephalin has at least some measure of opiate antagonist action, and the *in vivo* results described here indicate that Met⁵-enkephalin behaves as a mixed opiate agonist-antagonist. Our finding that

Leu⁵-enkephalin acts like morphine is compatible with *in vitro* data showing it to be a 'purer' agonist than Met⁵-enkephalin⁷. It may be that such mixed action of any opioid *in vivo* is simply the expression of the drug's relative ability both to displace endogenous opioid from opiate receptor sites and to act there itself. Failure of intraventricular naloxone (which aggravated pain but produced no signs of narcotic withdrawal) to behave identically with Met⁵-enkephalin might be due to differences in affinity between these substances for opiate receptors at sites differentially involved in the two behaviours. Aggravation of pain with parenteral naloxone at low doses has already been described^{21,22}.

Various endogenous molecular species^{1-7,23,24} may interact with the opiate receptor in response to noxious stimuli. Prominent among these is the C-fragment of β -lipotropin²⁵. The C-fragment molecule is 35 times as potent as Met⁵-enkephalin in displacing ³H-dihydromorphine; it has been reported to cause prolonged analgesia after infusion into the third ventricle of cat, and to show an analgesic potency, on a molar basis, 200 times that of morphine²⁵. Our present findings with intraventricular Met⁵-enkephalin and naloxone suggest that several endogenous opioids may be competing at multiple sites when mobilised during painful stimulation.

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Enkephalin inhibits firing of myenteric neurones

ENDOGENOUS ligands for the opiate receptor have recently been described. The first to be characterised were methionine-enkephalin and leucine-enkephalin, pentapeptides isolated from porcine brain by Hughes *et al.* and subsequently synthesised¹. The enkephalins are also found in high concentrations in the myenteric plexus of the guinea-pig ileum^{2,3}, a neuronal network which is widely used as a model in investigations of narcotic action⁴. In the myenteric plexus, narcotic analgesics inhibit the neuronal firing which can be recorded with a glass suction electrode^{5,6}. This action is stereospecific, is reversed by narcotic antagonists such as naloxone and is not dependent upon the presence of any synaptic input to the neurones⁶. The endogenous presence of enkephalin in brain prompted an examination of its effects when applied by iontophoresis to units whose activity was recorded extracellularly⁷⁻¹⁰. We have adopted an alternative approach in which known concentrations of enkephalin are applied to isolated ganglia of the myenteric plexus whilst recording neuronal activity. The results indicate that low concentrations of the enkephalins act on the myenteric neurones to inhibit their firing.

Ganglia of the myenteric plexus, adherent to the longitudinal muscle layer, were perfused in a Krebs saline solution. Activity was recorded from single neurones with a glass suction electrode of 30–50- μ m tip diameter. The myenteric neurones are not spontaneously active within the isolated ganglia, but recently we have shown that the firing recorded with a suction electrode is initiated in the cell soma probably as a consequence of mechanical contact with the recording electrode (North and Williams, in prepara-

tion). In these conditions recordings can be made from single neurones for periods of several hours. The present results are based on recordings from 80 units in 20 guinea pigs. Both methionine-enkephalin and leucine-enkephalin caused an immediate inhibition of neuronal firing (Fig. 1). The depression of spike firing was dose-related; neuronal firing was often completely stopped by 100 nM Met⁵-enkephalin, and markedly inhibited by 10 nM. The firing rate increased immediately on washing with drug-free Krebs solution; in the majority of neurones the washout was associated with a transient but marked "rebound" increase in the rate of discharge (Fig. 1).

Met⁵-enkephalin was approximately five times more potent than Leu⁵-enkephalin when tested on the same unit (Fig. 1). Met⁵-enkephalin was five to ten times more potent than morphine or normorphine. When Met⁵-enkephalin is used to inhibit the nerve-mediated contractile response of the longitudinal muscle layer, it is approximately equipotent with morphine¹¹. However, in those experiments there is a relatively large piece of tissue whereas in our experiments there are only a few isolated ganglia. It is possible that the potency of enkephalin appears to be less in the experiments on the complete longitudinal muscle strip because a large proportion of the dose administered is bound nonspecifically and subsequently destroyed by peptidase. Further support for this notion derives from experiments in which we applied enkephalin for periods of several minutes. When the whole strip is used, the contractile response of the muscle begins to recover after about 1 min despite the continued presence of the enkephalin. However, the firing rate of a single neurone remained depressed for as long as 20 min when the tissue was perfused by a solution containing Met⁵-enkephalin (Fig. 1). There was no evidence of tachyphylaxis. The action of enkephalin was reversed or prevented by a tenfold lower concentration of naloxone (Fig. 2). this concentration of naloxone applied alone was

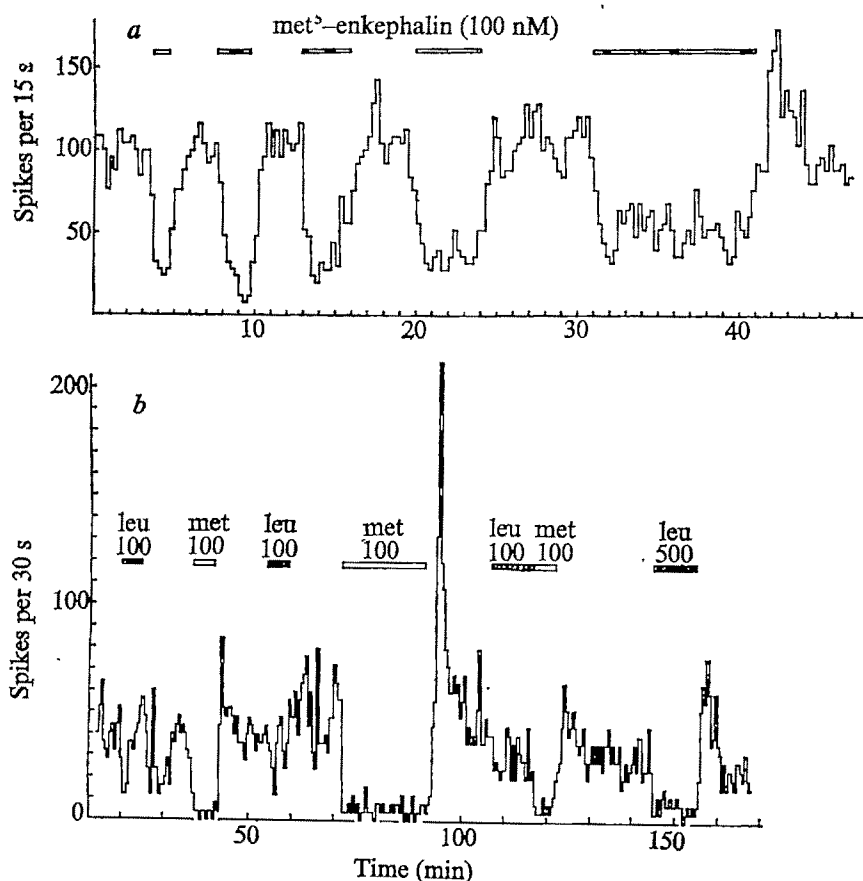
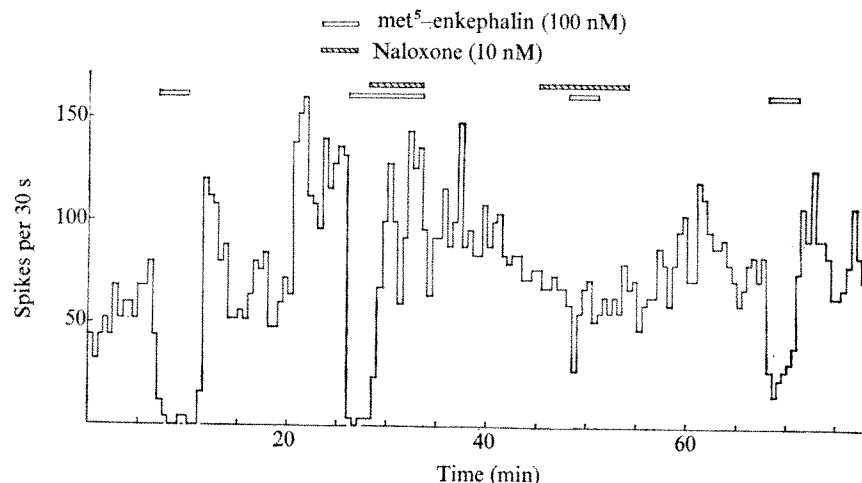


Fig. 1 Inhibition of firing of myenteric neurones by enkephalin. An isolated ganglion of the myenteric plexus was perfused with Krebs solution at 37 °C (solution composition (mmol l⁻¹): NaCl, 117; KCl, 4.7; CaCl₂, 2.5; MgCl₂, 1.2; NaHCO₃, 25; NaH₂PO₄, 1.2; glucose, 11.5; gassed with 95% O₂/5% CO₂). During the periods indicated by the horizontal bars the solution which perfused the tissue contained Met⁵-enkephalin or Leu⁵-enkephalin. *a*, Periods of exposure to Met⁵-enkephalin were 1, 2, 3, 4 and 10 min. In each case there was a marked inhibition of neuronal firing which reversed rapidly when the enkephalin application was stopped. *b*, A similar experiment on a different unit. Leu⁵-enkephalin (100 nM) had only a small and variable effect on cell firing, whereas Met⁵-enkephalin (100 nM) inhibited firing almost completely. The effect of Met⁵-enkephalin (100 nM) was similar to that of Leu⁵-enkephalin (500 nM). Notice in both experiments that there was no evidence of tachyphylaxis during exposures as long as 10 or 20 min. In these and other experiments, the "rebound" increase in firing rate when the enkephalin was washed out was more pronounced with longer periods of exposure.

Fig. 2 Effect of Met⁵-enkephalin and naloxone on the firing rate of a single myenteric neurone. During the periods indicated by the open and hatched bars, the solution which perfused the tissue contained Met⁵-enkephalin (100 nM) and naloxone (10 nM) respectively. Application of Met⁵-enkephalin for 3 min produced a complete inhibition of firing. This inhibition was rapidly reversed by changing to a solution which contained both Met⁵-enkephalin and a tenfold lower concentration of naloxone. In other experiments (for example Fig. 1) we have shown that the firing rate remains depressed by Met⁵-enkephalin even during prolonged applications. Naloxone itself was without significant effect on the firing rate, but it almost completely prevented the action of Met⁵-enkephalin. The final application of Met⁵-enkephalin to this neurone was probably somewhat reduced by the residual presence of some naloxone.



without effect on the firing rate. This finding substantiates the view that the enkephalin is acting on opiate receptors.

The technique which we have used offers certain advantages over the iontophoretic application of enkephalin *in vivo*. The precise concentration of enkephalin at the neurone is known, and degradation by tissue peptidase is largely avoided. Furthermore, as the cell firing is not dependent on synaptic input (ref. 6 and R.A.N. and J.T.W., in preparation), the enkephalins must be acting directly upon the neurone whose activity is recorded. The inhibition of firing by opiates was postulated to be due to a membrane hyperpolarisation⁶, and such an effect of morphine has recently been demonstrated by intracellular recording^{12,13}. The inhibition of neuronal firing by enkephalin may also be based on a membrane hyperpolarisation (R.A.N., unpublished). Inhibitory synaptic potentials have not been recorded in the myenteric plexus, and the role of enkephalin in this tissue remains obscure. On the other hand, if enkephalin inhibits the firing of central neurones by a similar mechanism then its candidacy as inhibitory transmitter would be supported.

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Gonadotropin-releasing hormone surge in pro-oestrous rats

It has generally been accepted that the pre-ovulatory surge of luteinising hormone (LH) is caused by an increased release of gonadotropin-releasing hormone (GnRH) from nerve terminals in the median eminence. So far, however, estimations of the concentrations of GnRH in hypophyseal portal vessel blood by bioassay¹ or radioimmunoassay^{2,3} have failed to demonstrate a surge of GnRH before or during the spontaneous surge of LH. The anaesthetics used (urethane^{1,3} and sodium pentobarbitone²) block ovulation, and, therefore, are likely to block or truncate a surge of GnRH, if it occurs. We have re-examined this problem using Althesin (Glaxo, Middlesex) an anaesthetic which consists of 9 mg alphaxalone (3 α -hydroxy-5 α -pregnane-11,20-dione) and 3 mg alphadolone acetate (21-acetoxy-3 α -hydroxy-5 α -pregnane-11,20-dione) per ml of isotonic aqueous vehicle and show that it does not block the spontaneous LH surge or ovulation. Previous studies on the cat⁴ suggested that, although it can provide adequate analgesia, Althesin does not interfere with various forebrain functions.

All experiments were carried out on female Wistar rats, 200-300 g body weight, which were maintained under controlled lighting (lights on 0500-1900) and temperature (22 °C), and exhibited regular 4-d oestrous cycles. The animals were given free access to Diet 41B (F. Dixon & Sons, Ware) and tapwater. A dose of 0.5-0.6 ml Althesin per 100 g body weight, administered intraperitoneally, produced adequate analgesia (no somatomotor response to painful stimuli) for 2.0-3.5 h. Supplementary doses of 0.25-0.5 ml could be given at 2.0-h intervals without risk of fatality.

To test the effect of Althesin on ovulation and LH release, 14 rats were anaesthetised at 1330 on the day of pro-oestrus, and blood samples (0.3 ml) were taken from the external jugular vein either at 1800 ($n = 8$) or 5, 30 and 60 min ($n = 6$) after injection. Inspection of the oviducts on the following morning (expected day of oestrus) revealed that ovulation occurred in all the animals (mean \pm s.e.m. ova count per animal = 8.6 ± 1.0). The mean \pm s.e.m. concentration of LH, estimated by radioimmunoassay^{5,6}, in the samples taken at 1800 was 25.0 ± 8.8 ng NIH-LH-S18 ml⁻¹. Although lower than those in unanaesthetised animals or in animals anaesthetised immediately before blood withdrawal⁶⁻⁸, these results showed that, in contrast to sodium pentobarbitone and urethane, Althesin administered before the 'critical period' of pro-oestrus⁹ did not block the spontaneous surge of LH or ovulation. The plasma LH concentrations of the blood samples taken 5, 30 and 60 min after Althesin injection were 3.4 ± 0.5 , 3.1 ± 0.3 and 3.4 ± 0.3 ng ml⁻¹ respectively, indicating that Althesin does

Table 1 Mean (\pm s.e.m.) concentrations of GnRH in pituitary stalk blood and volumes of collections

Group	Stage and time (h) of cycle		No. of animals	No. of samples in which GnRH detectable*	Conc. GnRH (pg ml ⁻¹)	Blood volume (μ l per 30 min)
1	Metoestrus	0900–1100	23	20	25 \pm 3	513 \pm 56
2	Metoestrus	1600–1830	22	21	34 \pm 7	301 \pm 40
3	Dioestrus	0900–1100	26	18	23 \pm 3	475 \pm 40
4	Dioestrus	1600–1830	23	20	20 \pm 2	363 \pm 28
5	Pro-oestrus	0800–0930	16	11	26 \pm 4	382 \pm 41
6	Pro-oestrus	0930–1030	18	17	31 \pm 3	605 \pm 123
7	Pro-oestrus	1030–1130	21	21	27 \pm 2	461 \pm 59
8	Pro-oestrus	1130–1500	52	43	24 \pm 2	371 \pm 30
9	Pro-oestrus	1500–1600	16	13	49 \pm 11	296 \pm 72
10	Pro-oestrus	1600–1630	12	12	77 \pm 30	448 \pm 112
11	Pro-oestrus	1630–1700	14	14	80 \pm 38	418 \pm 80
12	Pro-oestrus	1700–1730	15	15	85 \pm 34	384 \pm 70
13	Pro-oestrus	1730–1800	14	13	151 \pm 75	327 \pm 63
14	Pro-oestrus	1800–1830	9	9	121 \pm 77	264 \pm 50
15	Pro-oestrus	1830–2000	16	14	45 \pm 4	556 \pm 91
16	Pro-oestrus	2000–2230	27	21	33 \pm 3	482 \pm 65
17	Pro-oestrus	2230–2400	18	9	37 \pm 8	440 \pm 65
18	Oestrus	0000–0200	22	15	66 \pm 13	389 \pm 50
19	Oestrus	0200–0400	18	8	35 \pm 7	319 \pm 37
20	Oestrus	0900–1100	22	20	26 \pm 3	395 \pm 39
21	Oestrus	1600–1830	34	33	18 \pm 1	419 \pm 45

*For definition see text.

Significance of differences between means as determined by analysis of variance and multiple range test:

 $P < 0.05$: group 10 compared with groups 4 and 21; group 11 compared with groups 3, 4, 8 and 21; group 12 compared with groups 1, 3–5, 7, 8, 20 and 21; group 14 compared with group 18. $P < 0.01$: group 13 compared with group 12. $P < 0.005$: group 13 compared with groups 10 and 11; group 14 compared with groups 9, 15 and 17. $P < 0.001$: group 13 compared with all groups except 10–12 and 14; group 14 compared with groups 1–8, 16 and 19–21.

not stimulate LH release. A possible progesterone-like action of Althesin was also tested (for rationale see ref. 10) in long term ovariectomised rats which had been given 20 μ g oestradiol benzoate, subcutaneously, 72 h before the administration of either 0.5 ml Althesin per 100 g, 2.5 mg progesterone or 0.5 ml arachis oil. Under ether anaesthesia, 5 h later, a blood sample was taken from the external jugular vein. The mean (\pm s.e.m.) plasma LH concentrations were 18.2 ± 6.2 , 35.8 ± 13.8 and 147.5 ± 35.4 ng ml⁻¹ in animals treated with oil ($n = 6$), Althesin ($n = 6$) and progesterone ($n = 7$), respectively. There was no significant difference between the mean plasma LH concentration in the oil, compared with the Althesin-treated group; however, the concentration in the progesterone-treated animals was significantly greater than that in oil- ($P < 0.005$) or Althesin- ($P < 0.02$) treated animals.

The concentration of GnRH in pituitary stalk blood was studied in 132 animals anaesthetised at frequent intervals during the oestrous cycle. The pituitary stalk was exposed according to Worthington¹¹ and Fink and Jamieson³. Special care was taken to ensure haemostasis; in particular, damage to the tuberoinfundibular artery was avoided whenever possible. Blood was collected during 3–6 consecutive 30-min periods and plasma GnRH concentration was determined by double antibody radioimmunoassay^{3,12,13}. The lower limits of sensitivity of

these assays for 100- μ l samples (mean c.p.m. -2 s.d. of the total bound tubes) ranged from 6.2 to 15.8 pg GnRH ml⁻¹. These values were assigned to the relatively few samples (see Tables 1 and 2) in which the concentration of GnRH was below the limit of sensitivity of the assay. For statistical purposes, the GnRH concentrations of the 30-min collections between various times of the cycle were grouped together to determine the mean concentrations shown in Table 1. Details of the GnRH concentrations in the 30-min collections are shown in Fig. 1. There were no appreciable differences between the mean GnRH concentrations on the days of metoestrus and dioestrus, and between 0800 and 1500 of pro-oestrus. Concentrations then rose to reach a value at 1730–1800 which was significantly greater than that at any other time except between 1800 and 1830. The decline in concentration which occurred after 1830 of pro-oestrus was interrupted by a small peak between 2230 of pro-oestrus and 0200 of oestrus. There were no significant differences between the volumes of the collections between 1030 and 1830 of pro-oestrus and between 2230 of pro-oestrus and 0200 of oestrus. Table 2 shows that the GnRH concentration of pituitary stalk blood collected during Althesin anaesthesia between 1600 and 1830 of pro-oestrus was significantly greater ($P < 0.05$) than the concentration in stalk blood collected at the same time from animals anaesthetised with sodium pentobarbitone. The

Table 2 Mean (\pm s.e.m.) concentrations of GnRH in pituitary stalk, 'pharyngeal' and jugular venous plasma, and volumes of collections

Source of plasma*	No. of samples	No. of samples in which GnRH detectable†	Concentration of GnRH (pg ml ⁻¹)	Blood volume (μ l per 30 min)
Stalk plasma	64	63	102 \pm 23	374 \pm 35
Althesin anaesthesia				
Stalk plasma	23	17	19 \pm 1	459 \pm 38
Sodium pentobarbital anaesthesia				
Pharyngeal plasma	31	14	25 \pm 2	450 \pm 61
Althesin anaesthesia				
Jugular venous plasma	40	6	19 \pm 1	c.a. 500
Althesin anaesthesia				

*All samples were obtained between 1600–1830 on day of pro-oestrus.

†As for Table 1.

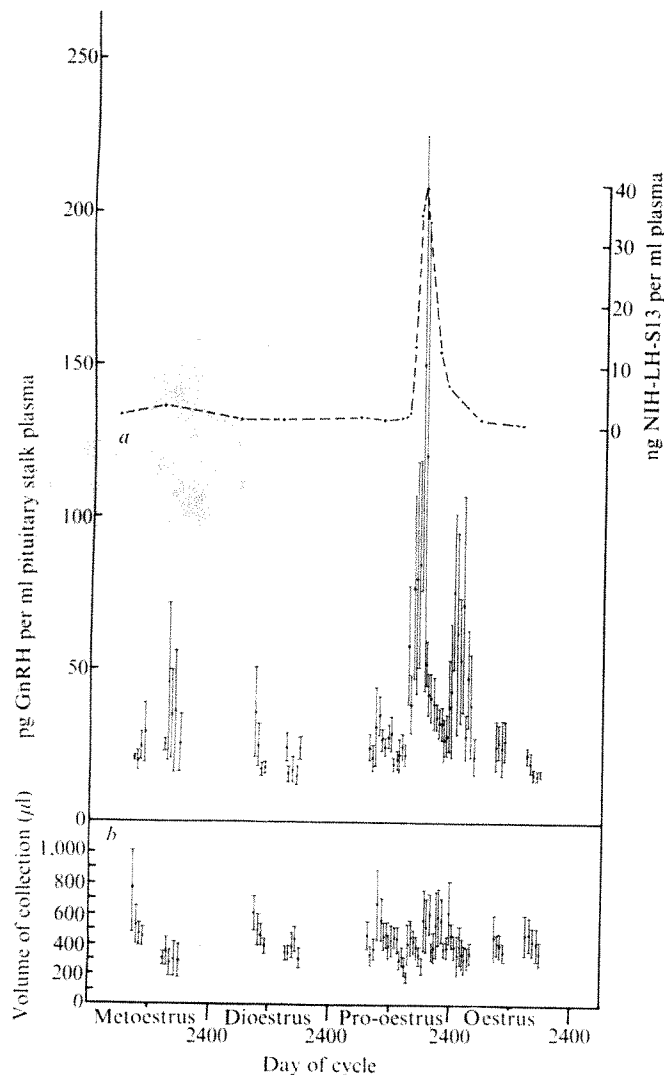


Fig. 1 Mean (\pm s.e.m.) concentrations of GnRH (a) and volumes (b) of 30-min collections of pituitary stalk blood. Most means are based on 5–15 samples, a few on 3–4 samples. The mean concentrations of plasma LH (---) (data from ref. 6, with permission) are shown for comparison.

difference between the volumes of the collections was not significant. The concentration of GnRH in stalk plasma collected under Althesin was also greater ($P < 0.05$) than that in blood collected from the pharyngeal mucosa (for method see ref. 3) and in blood withdrawn from the external jugular vein ($P < 0.01$).

Comparison of the present data with peripheral plasma LH concentrations in rats of the same strain similarly maintained (Fig. 1) suggests that the LH surge begins shortly after the GnRH surge. After the peak, plasma LH concentrations fall in parallel with stalk plasma GnRH concentrations. The large variation in GnRH concentration, especially between 1600 and 1830 (values ranging from undetectable to 950 pg ml^{-1}) may be due to a pulsatile release of this hormone. Similar concentrations of GnRH in pituitary stalk blood have also been found around the mid-cycle of rhesus monkeys anaesthetised with phencyclidine hydrochloride¹⁴.

The smaller peak of stalk plasma GnRH concentration between 2230 of pro-oestrus and 0200 of oestrus may be related to the pre-ovulatory surge of plasma FSH which continues to rise after the cessation of the LH surge and reaches a peak at about 0500 of oestrus^{6,15}. This peak of GnRH concentration also occurs at about the time of maximal sexual receptivity¹⁶ and immediately precedes ovulation (0200–0400 of oestrus).

It has been established that in the rat and other spontaneous ovulators including man, there is a massive increase in the responsiveness of the anterior pituitary gland to GnRH which occurs before and during the pre-ovulatory surge of LH^{6,17}. The present results reveal the importance of this increase in pituitary response, for, even allowing for a twofold dilution of pituitary stalk collections by blood from sources other than the hypothyseal portal vessels¹, the mean concentrations of GnRH between 1600 and 1830 on the day of pro-oestrus are relatively low compared with a level of about $3,000 \text{ pg ml}^{-1}$ in the peripheral circulation, which is achieved 1 min after the intravenous injection of 50 ng synthetic GnRH per 100 g body weight¹³ (minimal ovulatory dose⁶). Intravenous infusion and multiple injection studies (ref. 13 and N.M.S., S.A.C. and G.F., unpublished) have shown that plasma GnRH concentrations of $100\text{--}400 \text{ pg ml}^{-1}$ are capable of producing a surge of LH on the afternoon of pro-oestrus provided that the pituitary is exposed to these levels for at least 30 min. Similar concentrations of plasma GnRH produce only a slight rise in plasma LH on the afternoon of dioestrus¹³. Thus, without the 50-fold increase in pituitary responsiveness to GnRH between 1330 on the day of dioestrus and 1730 on the day of pro-oestrus⁶ the spontaneous LH surge may not occur. The priming effect of GnRH¹³ which is activated by the increased secretion of GnRH during the afternoon of pro-oestrus may be responsible for ensuring that the peaks of responsiveness and the GnRH surge coincide.

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Synthesis of luteinising hormone releasing factor and thyrotropin-releasing factor in glutamate-lesioned mice

The arcuate nucleus (ARC) of the mammalian hypothalamus is thought to regulate tonic luteinising hormone (LH) secretion from the anterior pituitary¹. But the immunohistochemical demonstration of luteinising hormone

releasing factor (LHRF) in nerve fibres but not cell bodies of the ARC^{2,3} and the marked reduction in LHRF content after hypothalamic deafferentation⁴, suggest that the ARC is not a centre for LHRF synthesis. To clarify this issue we have used monosodium glutamate, a neurotoxin which when administered in a low dose to young animals selectively destroys ARC neurones while leaving intact axons passing through⁵⁻⁷. If the ARC perikarya synthesise LHRF, a marked reduction in the immunohistochemical staining and content of hypothalamic LHRF should be evident in glutamate-lesioned animals. Because thyroid function is unaffected by glutamate⁸, we examined brain tissue for thyrotropin releasing factor (TRF) content as a control for the LHRF studies.

Ten-day-old CD1 albino mice (Charles River) received a single injection of glutamate (subcutaneous 18 mmol per kg body weight in a 10% aqueous solution), and their litter mate controls received an equal dose of NaCl. All animals were decapitated 96 h later. By this time, orthograde degeneration and phagocytosis of tubero-infundibular axons would be expected to be complete in the median eminence (ME) and stalk median eminence (SME)⁹. Brains from seven experimental and five control animals were removed rapidly. The hypothalamus was excised and extracted in 1 ml of 2N acetic acid. The remainder of the brain was extracted in 2 ml of 2 N acetic acid. An aliquot was removed for measurement of protein concentration¹¹. An aliquot (0.3 ml) of the remainder was freeze dried, redissolved in 0.01 M phosphate-buffered saline, pH 7.4, and assayed for LHRF using a radioimmunoassay with a sensitivity of 2 pg (I.M.D.J. and S. Reichlin, in preparation). Briefly, LHRF was labelled with ¹²⁵I using chloramine T and purified on a column of Sephadex G-25. The sample, ¹²⁵I LHRF and anti-LHRF at a titre of 1:96,000 were incubated for 48 h, and bound hormone was separated from free hormone with dilute charcoal. Antibody to LHRF was raised in a rabbit immunised with synthetic LHRF conjugated to bovine thyroglobulin, by means of *bis*-diazotised benzidine, similar to that used to develop anti-TRF¹². The LHRF antiserum reacted with the LHRF decapeptide as well as with certain C-terminal fragments (nonapeptide through pentapeptide). No cross-reaction occurred with the deamidated free acid form of LHRF, TRF, somatostatin, vasopressin, thyroid or anterior pituitary hormones. Hypothalamic and extrahypothalamic TRF in the same specimens were measured by a sensitive and specific radioimmunoassay for TRF¹².

In glutamate-treated animals there was a marked reduction in the number of neuronal cell bodies within the ARC (Fig. 1). Reaction product localising immunoreactive LHRF, however, was abundant and seen throughout the rostro-caudal extent of the ME and in the upper SME, as well as in the organum vasculosum of the lamina terminalis. Similar to the distribution described in adult mammals^{2,13}, immunoreactive LHRF in glutamate-lesioned mice tended to accumulate at the tubero-infundibular sulci, and to become more intensely concentrated in caudal regions just proximal and just distal to the SME. There was no difference in staining pattern or intensity compared with

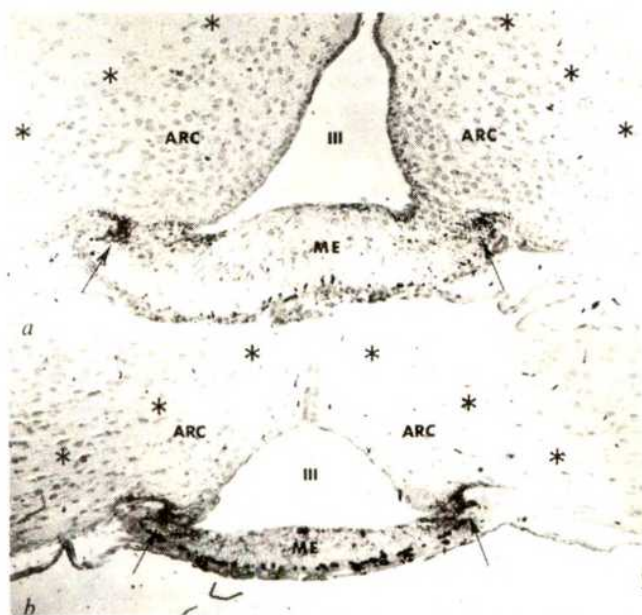


Fig. 1 Light micrograph ($\times 46$) of a frontal section through the basal hypothalamus comparing the experimental (*b*) with the control (*a*) and demonstrating intense reaction product of immunoreactive LHRF at the tubero-infundibular sulci (arrows). Note marked reduction ($> 80\%$) in the size of the arcuate nucleus (ARC). ME, median eminence; III, third ventricle.

controls. In no instance was immunoreactive LHRF seen within ARC perikarya of either NBF or Bouin's fixed tissues.

Hypothalamic LHRF content of glutamate-treated mice (mean \pm s.e.m.), $1,275 \pm 106$ pg LHRF per hypothalamus ($7,734 \pm 1,389$ pg LHRF per mg protein), was similar to that seen in controls, $1,371 \pm 343$ pg per hypothalamus ($7,493 \pm 2,427$ pg LHRF per mg protein) (Table 1). In both experimental and control animals, extrahypothalamic LHRF content was very low, being at or just below the limits of sensitivity of the assay. Like LHRF, hypothalamic TRF content did not differ significantly between glutamate-treated ($2,178 \pm 165$ pg TRF per hypothalamus) and control ($2,532 \pm 159$ pg TRF per hypothalamus) animals. But TRF was readily measurable in considerable quantity in the extrahypothalamic brain of both groups (glutamate: $3,601 \pm 178$ pg TRF, control: $3,417 \pm 144$ pg TRF). These differences were not significant.

This study provides evidence that glutamate-sensitive ARC neurones do not give rise to the LHRF immunoreactive pathway, for glutamate-induced lesions, which destroyed most ARC neurones, neither altered the quantity and distribution of immunohistochemically demonstrable LHRF in the ME nor decreased total hypothalamic LHRF content. Therefore large concentrations of LHRF in the ARC, as determined by Palkovits *et al.*¹⁴, probably reflect the content of dense cored vesicles within fine calibre axons that course among ARC perikarya. Such LHRF-containing axons may also explain observations^{2,3} that immunoreactive LHRF fibres can be traced to the ARC. Their origin from

Table 1 Effect of monosodium glutamate on the hypothalamic and extrahypothalamic content of LHRF and TRF in mice (mean \pm s.e.m.)

	LHRF		TRF	
	pg per tissue	pg per mg protein	pg per tissue	pg per mg protein
Hypothalamus				
Glutamate	$1,275 \pm 106$	$7,734 \pm 1,389$	$2,178 \pm 165$	$12,914 \pm 1,637$
Control	$1,371 \pm 343$	$7,493 \pm 2,427$	$2,532 \pm 159$	$13,393 \pm 2,297$
Extra-hypothalamic brain				
Glutamate	< 66	< 18.5	$3,601 \pm 178$	829 ± 68
Control	< 66	< 16.3	$3,417 \pm 144$	840 ± 57

ARC perikarya, however, need not be imposed. Furthermore, many workers have failed to confirm^{2,13,15-17} reports that mammalian ARC perikarya are immunoreactive for LHRF¹⁸⁻²⁰, and it has been suggested that perikaryal staining described in these ARC is due to a nonspecific tissue-immunoglobulin reaction^{2,17}.

The data reported here suggest that the LHRF in the ARC region of mice is located in dense cored vesicles of axons passing through the nucleus, though its presence within the small number of ARC perikarya, which are not glutamate-sensitive in the conditions of the experiment, is not excluded. Further study of the origin of the immunohistochemically demonstrable LHRF-positive fibres in the ME and upper SME is needed.

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Impairment of luteinising-hormone release following oestrogen administration to hyperprolactinaemic ewes

THE antagonistic role of prolactin during the post-partum period seems well established in many species¹⁻³. But mechanisms by which prolactin may act are not yet known. There is some evidence that prolactin may exert its inhibitory function at the ovarian level^{4,5}, but an eventual action on the hypothalamo-hypophysial axis cannot be excluded. Experiments described here have been designed to resolve this problem.

The responsiveness of the pituitary to luteinising hormone releasing hormone (LHRH) injections, was studied in eight suckling 'Préalpes du Sud' ewes. All the animals were spayed in October, 1 d after parturition, to eliminate the variable of ovarian steroid feedback. Four of the ewes were given 1 mg 2-Br- α -ergocryptine (CB 154, Sandoz) intramuscularly, twice daily from

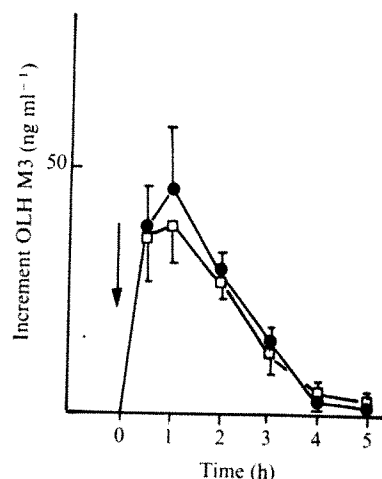


Fig. 1 LH increments in spayed ewes after intravenous injection of 200 μ g LHRH (arrow): \bullet , control ewes; \square , TRH-treated animals. (Mean values \pm standard error. Four animals in each group.)

the time of parturition to day 60 *post partum* to suppress prolactin levels⁶. The other four animals served as untreated controls with high prolactin levels. Ovine prolactin and LH were measured by radioimmunoassay^{7,8}.

Both groups were injected once a week for eight weeks with an intravenous bolus of 200 μ g synthetic LHRH (Hoechst) and an LH release was always observed in all animals. The responses were very weak on the day of parturition (before castration), but increased progressively during the first four weeks *post partum* and remained constant thereafter. Since the pattern of LH release was indistinguishable in the treated and control group, it was concluded that high prolactin levels do not modify the ability of the pituitary to discharge LH in response to LHRH.

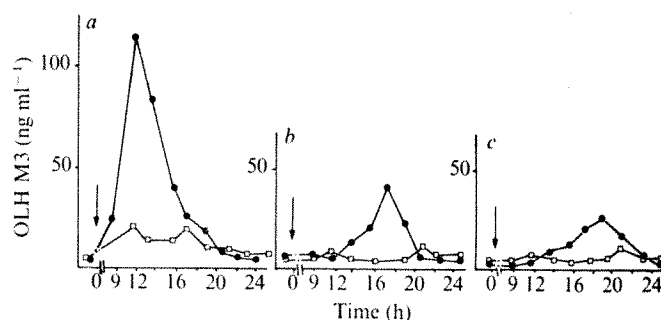


Fig. 2 Inhibition during hyperprolactinaemia of LH release attributable to oestradiol-17- β in three spayed ewes. \bullet , control ewes; \square , TRH-treated animals. Arrows, intramuscular oestradiol-17- β : a, 25 μ g; b, 25 μ g; c, 12 μ g.

A similar experiment was performed on a group of spayed ewes that were not lactating. Intravenous LHRH (200 μ g) was given twice at 3-d intervals, on the first occasion during a control infusion with physiological saline, and the second during a time when prolactin levels had been artificially elevated by repeated infusions of thyrotropin releasing hormone (TRH), 20 μ g, 12 times a day. This produced an elevated prolactin level 48 h before the LHRH stimulation, and mimicked the suckling-induced prolactin surges⁹. Once again, the LH response to exogenous LHRH was not modified by the hyperprolactinaemia (Fig. 1). These results are also in agreement with observations on women in hyperprolactinaemic amenorrhea¹⁰⁻¹², where the LHRH response was normal. Hyperprolactinaemia in the monkey may, however, render the pituitary less responsive to LHRH¹³.

To see whether the positive feedback effect of oestradiol on the hypothalamus was impaired by high prolactin levels, ewes castrated for more than three months received at 48-h intervals three

randomised intramuscular injections of oestradiol-17- β (12, 25, 50 μ g). The most effective dose in terms of gonadotropin release differed from one animal to another. This dose of oestradiol-17- β became ineffective after the same animal had been rendered hyperprolactinaemic with TRH infusions, as described previously (Fig. 2).

These findings, together with recent data from women^{14,15}, suggest that the positive feedback effect of oestradiol on LH release^{16,17} is greatly impaired by high prolactin levels. This is probably one of the ways in which suckling inhibits ovulation.

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Implantable artificial endocrine pancreas unit used to restore normoglycaemia in the diabetic rat

PARENTERAL insulin therapy has long been the primary form of treatment for patients with diabetes, but it does not prevent the chronic complications of diabetes, which involve retinopathy, neuropathy, nephropathy and vascular disease and cause the most morbidity and mortality of diabetic patients. One of the most promising alternative approaches involves transplantation of insulin-producing tissue. In animal models, sustained correction of diabetes has been accomplished by the transplantation of isolated pancreatic islets from donors syngeneic with the recipients^{1,2}. We report here experiments with an implant in which islet cells are placed outside a synthetic capillary (hollow fibre) through which smaller molecules, such as insulin, can diffuse freely. Using this device we have normalised the high glucose level of diabetic rats.

The capillary consisted of a polyvinyl chloride-acrylic copolymer (XM-50) fibre (Amicon) with a 450- μ m internal diameter which had a nominal molecular weight cutoff of 50,000. A 2.8-cm long fibre was connected by epoxy resin at both ends with 5-cm pieces of radiopaque intravenous catheter with a 0.81-mm internal diameter and 1.2-mm outside diameter (Venocath-16, Abbott). The fibre was inserted into 3 cm of polyethylene shell (Intramedic Tubing, PE320, Clay Adams, B.D. and Co., Parsippany, New Jersey) and the implantable artificial capillary unit was made by sealing the lumen of the polyethylene shell and the fibre at the fibre-catheter junction (Fig. 1).

Islets of Langerhans were isolated from male Wistar rats

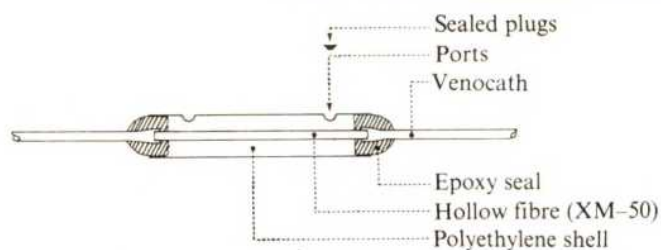


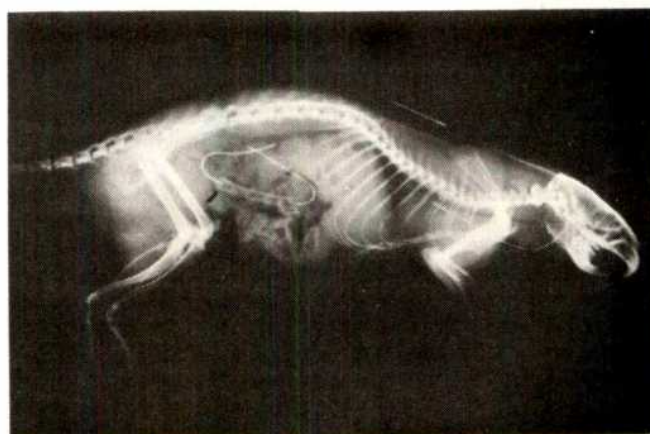
Fig. 1 Diagram to show the construction of the implantable artificial endocrine pancreas.

(310-330 g) by collagenase digestion³ and purified by Hypaque-Ficoll density gradient centrifugation⁴. Between 1,200 and 1,500 intact islets were suspended in Medium 199 supplemented with 10% foetal calf serum, penicillin (100 U ml⁻¹), streptomycin (0.1 mg ml⁻¹) and kanamycin (0.1 mg ml⁻¹) and were introduced into the artificial capillary unit through two 0.12-cm holes in the polyethylene shell. The holes were plugged with 'fitted rubber' tips (Critocap-J, Sherwood Medical Ind. Inc., St Louis) and sealed with epoxy resin.

This implantable artificial endocrine pancreas unit was perfused overnight with 125 ml of nutrient medium which was recirculated at a rate of 1.5-2.0 ml min⁻¹. Silastic tubing connecting the unit and the medium reservoir served as a gas exchanger. The circuit was operated at 37 °C in a humidified atmosphere of 5% CO₂ and 95% air.

We used male inbred Lewis rats, in which diabetes was induced by intravenous injection of streptozotocin (5.5 mg per 100 g) when they were between 300 and 320 g in body weight. An animal was defined as diabetic only when 24-h urine outputs were greater than 100 ml for at least 2 weeks and when the fasting blood glucose level was greater than 400 mg per 100 ml. Twenty-four hours before implantation, a radiopaque intravenous catheter (Venocath-16) was inserted into the jugular vein of the recipient diabetic rat to facilitate collection of blood samples, the infusion of heparin and the replacement of isogenic blood during the subsequent experiment. The catheter was kept open with saline containing heparin (10 U ml⁻¹). The diabetic rat was fasted for 4 h before implantation which was carried out under ether anaesthesia. An incision 3 cm long was made along the linea alba and the abdominal aorta was exposed. Heparin (50 U) was injected intravenously through the femoral artery. Two clamps were applied on the abdominal aorta between the renal artery and the ilio-lumbar artery. The implant was subsequently inserted directly into the abdominal aorta.

Fig. 2 Radiogram to show the positioning of the jugular vein catheter for blood sampling and the site of the implanted artificial endocrine pancreas as a vascular shunt in the abdominal aorta.



Samples of 0.4 ml of blood were withdrawn before the ether anaesthetic, every hour for the first 5 h after implantation and every other hour thereafter, for blood glucose and insulin determinations (by the Beckman glucose analyser and the insulin assay kit (Amersham Searle), respectively). Withdrawn blood was replaced with isogenic blood. To maintain the circulation through the implanted unit, the recipient was administered 50 U of heparin at 2-h intervals for the first 8 h and subsequently with 100 U of heparin every 4 h.

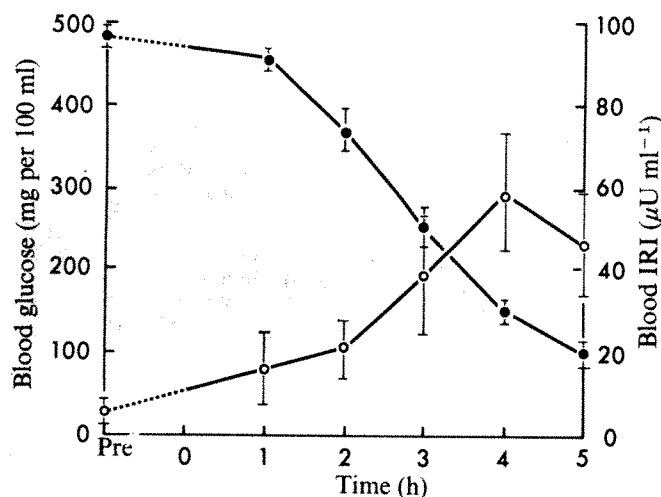


Fig. 3 Decline in blood glucose levels in diabetic rats after the implantation of an artificial endocrine pancreas. Pre indicates the sample taken before ether anaesthesia for the implantation procedure. Time 0 represents the connection of the artificial endocrine pancreas as a shunt in the circulatory system. The values expressed are the $\bar{X} \pm \text{s.e.}$ blood glucose and insulin levels of eight diabetic rats receiving such implants.

The positioning of the jugular vein catheter and the implanted artificial endocrine pancreas are shown radiologically in Fig. 2. In all the recipient streptozotocin-induced diabetic rats blood sugar levels started to decline within 2 h and achieved normoglycaemia between 4 and 5 h after implantation. There was a corresponding increase in blood insulin levels.

Figure 3 shows the trend of changes in blood glucose and circulating insulin in eight diabetic rats receiving

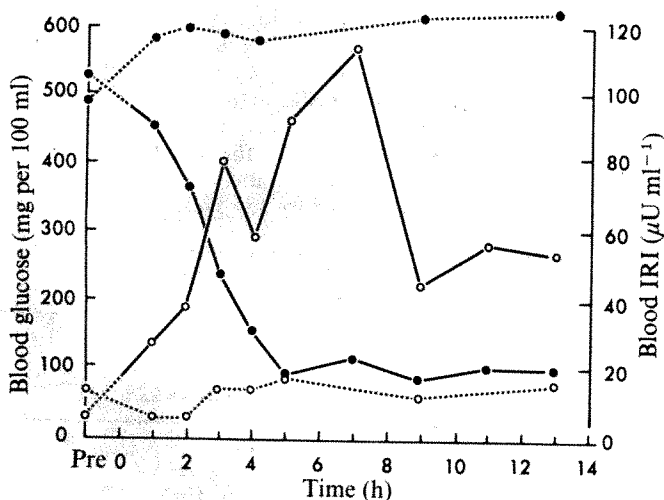


Fig. 4 The blood glucose and insulin levels in diabetic rats following the implantation of an artificial endocrine pancreas (—), and in diabetic rats following the implantation of an artificial capillary unit without islets (---). Each point represents the average value for two animals.

implants. The limited duration of study in this group was a consequence of excessive bleeding due to the effect of heparinisation. Figure 4 shows changes in insulin and blood sugar in two recipients which survived longer after implantation. Two diabetic control rats, given a capillary unit without islets, showed persistent hyperglycaemia and low insulin levels. There was clearly an improvement of hyperglycaemia and increased circulating insulin levels in diabetic rats after implantation with functional islets inside the artificial capillary unit.

We have shown already that the functional capacity of intact islets cultured in artificial capillary culture units *in vitro* is maintained for at least 97 d (ref. 5). Cultured islets responded to increased glucose concentration by increased insulin production throughout the culture period and the maintenance of the functional capacity of islets within the artificial capillary unit *in vivo* may also be anticipated. Although the *in vivo* studies were of limited duration, our results show that the implanted unit is sensitive to glucose and can produce sufficient insulin to normalise the high blood sugar levels in the diabetic rats within 4 h of implantation.

The potential importance of this approach is to provide functional pancreatic islets in the diabetic recipient which can supply insulin, glucagon and possibly other islet factors, in response to physiological needs and ultimately ameliorate insulin-requiring diabetes and perhaps prevent the development of complications. Islets within the unit may be protected from immune destruction by circulating host lymphocytes and antibodies, by virtue of the selective permeability of the molecular size of the synthetic capillaries. Thus, the artificial endocrine pancreas unit would mimic an immunologically privileged site. The system, if successful in the allogeneic rat, would have clinical potential.

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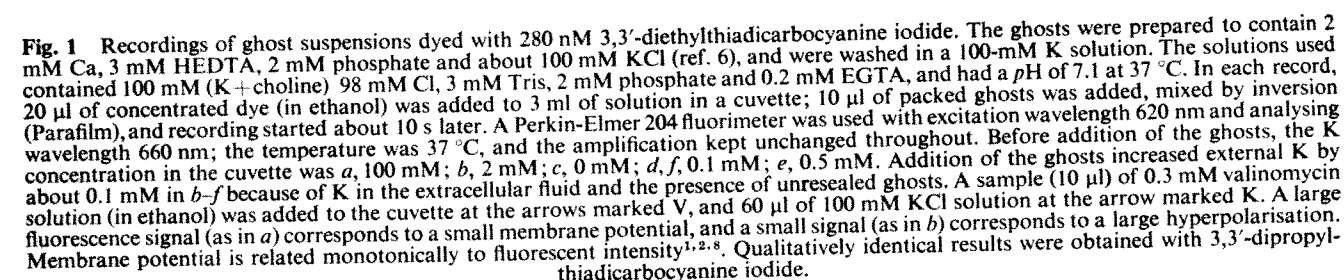
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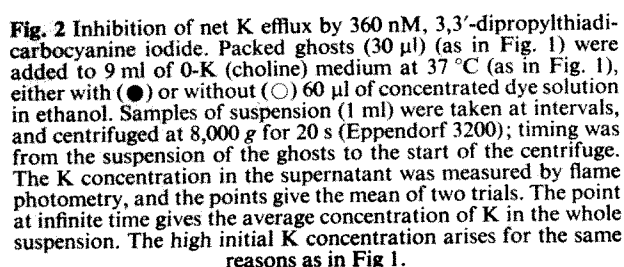
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Carbocyanine dyes inhibit Ca-dependent K efflux from human red cell ghosts

CARBOCYANINE dyes are fluorescent probes of membrane potential in red cells^{1,2}, and their application has been extended to Ehrlich ascites cells^{3,4} and phytoplankton⁵. I have used them to study the effect of potassium concentration gradients on the membrane potential of resealed ghosts containing Ca buffers. Intracellular Ca specifically renders these ghosts highly permeable to K (ref. 6). During this work, I discovered that 3,3'-dipropyl- and 3,3'-diethylthiadicarbocyanine iodide cause a progressive inhibition of K efflux at low external K concentrations, and that external K protects against the inhibitory effects of the dyes. The results suggest that the carbocyanine dyes are specific inhibitors of the Ca-dependent K transport system.



response was a large hyperpolarisation, followed by a rapid depolarisation to zero potential. Such a depolarisation might be caused by an equalisation of internal and external K, an increase in Cl permeability or a decrease in K permeability. Subsequent addition of $1\text{ }\mu\text{M}$ valinomycin (arrow V) caused a large hyperpolarisation, indicating that a large K gradient was still present. The (dipropyl) dye greatly reduces the rate of loss of K from the ghosts (Fig.2), so the depolarisation seen in Fig. 1c is caused by a decrease in K permeability. Figure 1d and e shows that a similar response is seen in solutions containing 0.1 mM K and 0.5 mM K, but the rate of depolarisation is reduced as the K concentration is raised. In Fig. 1f the ghosts were suspended in a 0.1-mM K solution, but the K concentration was increased by 2 mM during the depolarising phase (arrow K). This prevented further depolarisation, and the membrane potential was held at an intermediate value. Subsequent addition of valinomycin again produced a large hyperpolarisation. Similar experiments (not illustrated) showed that Rb and Cs ions are also able to prevent the depolarisation seen in low K solutions, whereas Na, Li, choline and tetraethylammonium ions are ineffective. This is the same cation selectivity as that of the Ca-dependent K transport system⁷. It seems likely that the depolarisations seen in Fig. 1 reflect a specific association between the dye and the K transport system, and the antagonistic effect of external K suggests that the reaction occurs at the external surface of the membrane. The specific nature of the interaction is confirmed by the lack of effect of the



	Ouabain -	Ouabain +
Dye -	0.94 ± 0.05	0.06 ± 0.02
Dye +	0.90 ± 0.03	0.04 ± 0.01

Washed human red cells (blood bank) were suspended at 37 °C, haematocrit 0.7%, in a solution containing 150 mM choline Cl, 8 mM Tris Cl, 2 mM Tris phosphate, 5 mM glucose and 0.16 mM ^{42}KCl (pH 7.3). When present, ouabain was at 70 μM and the dye at 300 nM. For each condition, samples were taken in triplicate at 0, 20, 40 and 60 min, centrifuged, and the cells washed three times in ice-cold incubation medium lacking ^{42}K . The influxes (in mmol per l cells h^{-1} , \pm s.d.) were calculated from plots of the radioactivity in the cells against time.

dye on the Na pump-catalysed influx of K into red cells suspended in a 0.16-mM K medium (Table 1).

The experiments illustrated in Figs 1 and 2 monitor a changing situation during the onset of inhibition. Not only is the degree of inhibition increasing, but the internal and external dye concentrations are changing during this period⁸. In spite of this, a rough dose-response curve (Fig. 3), constructed from a series of observations of K efflux rates, shows 50% inhibition of K efflux by about 20–50 nM 3,3'-diethylthiadicarbocyanine iodide at 0.1 mM external K. This is likely to be an overestimate of the half-inhibition constant because the measurements were made during the onset of inhibition, rather than at equilibrium.

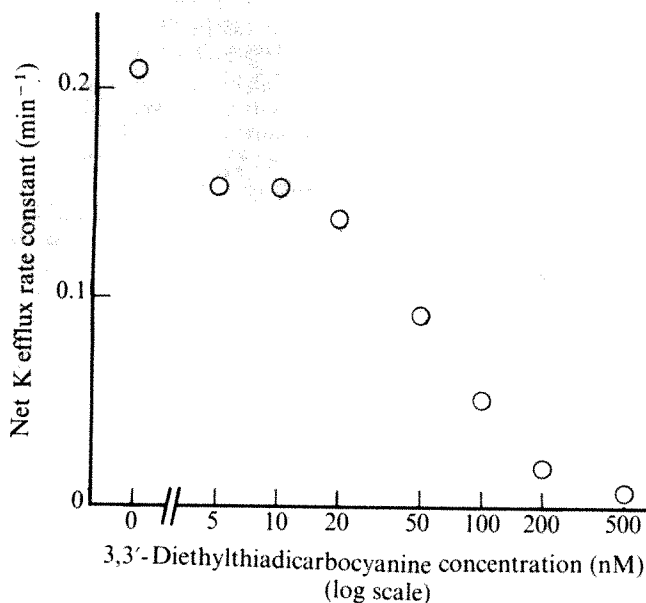


Fig. 3 Dose-response curve for the inhibition of net K efflux by 3,3'-diethylthiadicarbocyanine iodide. Each point gives the weighted mean of two determinations, from measurements made as in Fig. 2. To make a quantitative estimate of the rate of K efflux it was assumed (incorrectly) that external K would approach its equilibrium value exponentially, and the rate constants obtained from the slopes of straight lines fitted to plots of log (fraction of K remaining in ghosts) against time.

Preliminary experiments have also shown that, at fixed dye concentrations of 400 nM, 3,3'-diethylthiadicarbocyanine iodide and 3,3'-diethylthiatricarbocyanine iodide are as effective as 3,3'-diethyl- and 3,3'-dipropylthiadicarbocyanine iodide in inhibiting net K efflux from Ca-containing ghosts, whereas 3,3'-diethyloxycarbocyanine iodide and 3,3'-diethyl-9-ethyloxycarbocyanine iodide are less effective, and 3,3'-diethylthiacyanine iodide, 1,1'-diethyl-2,2'-pyridylcarbocyanine iodide and 1,1'-diethyl-2,2'-carbocyanine iodide are only slightly effective or ineffective. 3,3'-Diethylthiadicarbocyanine iodide (250 nM) also inhibits the net K loss from intact human red cells treated with 0.1 mM Pb acetate⁹, or loaded with Ca by 2 h preincubation with inosine + iodoacetamide + CaCl₂ (refs 10, 11).

Two important conclusions should be drawn from these findings. First, these carbocyanine dyes should not be assumed to be inert probes of membrane potential, even at nanomolar concentrations. Second, and perhaps more positively, some carbocyanine dyes might prove very useful in the study of Ca-dependent K permeability systems, which seem to be widely distributed in nature^{12–14}. For example, they might be used to dissect ionic currents in voltage-clamp studies, or even as a marker during membrane purification.

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Intercellular junctions of frog skin epithelial cells

It has been suggested that junctional complexes between epithelial cells provide intercellular exchange of electrolytes^{1–3}, which will result in more or less coupled behaviour and functional homogeneity of neighbouring cells. In epithelia, such as frog skin, built up of several layers, electrolyte exchange through low-resistance junctions between the different cell layers would, in addition, allow participation of all layers in active transepithelial transport of ions and increase the amount of ATPase involved in transport. Na exchange between the different layers of the frog skin epithelium has recently been demonstrated in K⁺-depolarised Ouabain-poisoned skins⁴. The microelectrode experiments reported here show that low resistance connections between the different cell layers exist even under normal conditions.

Abdominal skins of *R. temporaria* and *R. esculenta* were punctured from the epithelial side perpendicular to the surface. The epithelium was properly attached to a copper grid at the corial side by a negative pressure of ~30 cm H₂O. Trans-epithelial potential difference (PD_{tr}) and short circuit current (I_{sc}) were measured using an automatic device which changed rhythmically between open circuit (o.c.) and short circuit (s.c.). During the o.c. periods, current pulses (5 μ A, 0.5 s) were applied transepithelially. The instantaneous change in PD_{tr} served to calculate the skin resistance ($R_t = \Delta PD_{tr}/I$). The microelectrodes (3 M KCl, $R = 4$ –8 M Ω , tip potential < 5 mV) were moved by a 1- μ m step motor drive (Narishige). To ensure that any structure once penetrated by the microelectrode could not be penetrated again, the microelectrode was moved only in the forward direction during the individual observation periods. The microelectrode recorded:

PD_{sc} = potential under s.c. conditions; PD_0 and PD_b = potential difference between respectively epithelial and corial side, and microelectrode under o.c. conditions ($PD_b = PD_0 + PD_{tr}$); R_0 = resistance between epithelial side and microelectrode calculated from the instantaneous change of PD_0 upon trans-epithelial injection of current. In a part of the experiments, R_0 was estimated from $(PD_{sc} - PD_0)/I_{sc}$ and R_t from PD_{tr}/I_{sc} . All electrical parameters were registered on a two-channel chart recorder. The epithelial bathing solution (Cl or SO₄ Ringer) could be exchanged in about 1 s. Na-free solution was prepared by substituting K for Na.

Impalement of two successive cell layers occurred in eight experiments by chance. Figure 1 shows the redrawn record of one observation. After penetration of dense layers, the microelectrode recorded: $PD_{sc} = -54$ mV, $PD_o = -1$ mV, $PD_b = -99$ mV and had passed 75% of R_i . Na-free epithelial perfusion produced a reversible change of PD_{sc} to -88 mV and PD_o to -44 mV, while PD_b remained essentially unchanged. Changes of R_i and R_o are quantitatively the same. According to recent investigations^{5,6} these data are typical for intracellular location of the microelectrode. Microelectrode advancement resulted in a spontaneous breakdown of the potentials to values close to those expected for the corial solution. Most likely, the microelectrode had entered an intercellular space between the epithelial cells. Part of the transepithelial resistance, however, was still in front of the microelectrode, suggesting a contribution of the intercellular spaces to R_i . The microelectrode entered an intracellular space again at 43 μ m. Potentials, resistance and response to Na-free epithelial perfusion were similar to those from the first cellular impalement. The view that a cell was punctured beneath that impalement at first and lost thereafter is supported by the fact that, in several hundred impalements of the frog skin epithelium, no cell could be repunctured. After spontaneous breakdown, a third jump to potentials of the same range as in the more outward cellular layers was observed at 52 μ m. They were stable for 20 s only, but might indicate impalement of a third cell layer before the microelectrode had passed the epithelium.

Amiloride (10^{-4} M) was used in a second impalement of the same skin to block Na entrance. The response of PD_{sc} and I_{sc} occurred without measurable delay (0.2 s) in the first and the second cell layer, also supporting the idea of electrical coupling, since alterations of the intracellular ion content would require periods of several seconds. Table 1 summarises the values of PD_{sc} , PD_o and R_o/R_i . No systematic difference exists between the two cell layers, suggesting good connection of their intracellular spaces. Although the resistance of the intercellular

junctions cannot be computed from these values (this would require impalement of several cells at given distances as performed in other epithelia^{1,3,7,8}), the order of magnitude can be estimated from a comparison of the resistances presented by the apical membrane (R_a) of the two successive layers. R_a can be calculated from

$$\frac{1}{R_i} = \frac{1}{R_a} + \frac{1}{R_a + R_b}$$

if R_b , the paracellular shunt resistance, is assumed to be measured by R_i after Amiloride (10^{-4} M) and the voltage divider ratio $PD_o/PD_b = R_a/R_b$ is used^{7,8}. The following values were obtained from the experiment, presented in Fig. 1 ($R_a = 6.8$ k Ω cm²): $R_a = 2.8$ k Ω cm² (control) and 8.0 k Ω cm² (Na free) in the first cell layer; $R_a = 3.0$ k Ω cm² (control) and 7.9 k Ω cm² (Na free) in the second layer. Appreciation of possible errors excludes the possibility that the difference in R_a , that is the resistance between the two layers, exceeds 0.3 k Ω cm². The resistance of the basolateral membranes (R_b) was considerably higher (0.9 k Ω cm² in control and 1.2 k Ω cm² with Na-free epithelial perfusion). Ion exchange between the different layers is thus less impeded than membrane passage and will provide distribution of Na through all cell layers. This must not exhibit characteristics of a single compartment since entrance across the outer border will be followed by fast distribution of Na within the cytoplasm of the first reacting cell layer, while passage into deeper layers requires penetration through—at least—space restricting junctional complexes. In addition, a certain fraction of the Na that has entered is already extruded into the intercellular spaces. This may explain why only one component during positive or negative current transients could be detected by Morel and Leblanc⁴ in transporting frog skins. But if Ouabain blocked Na extrusion

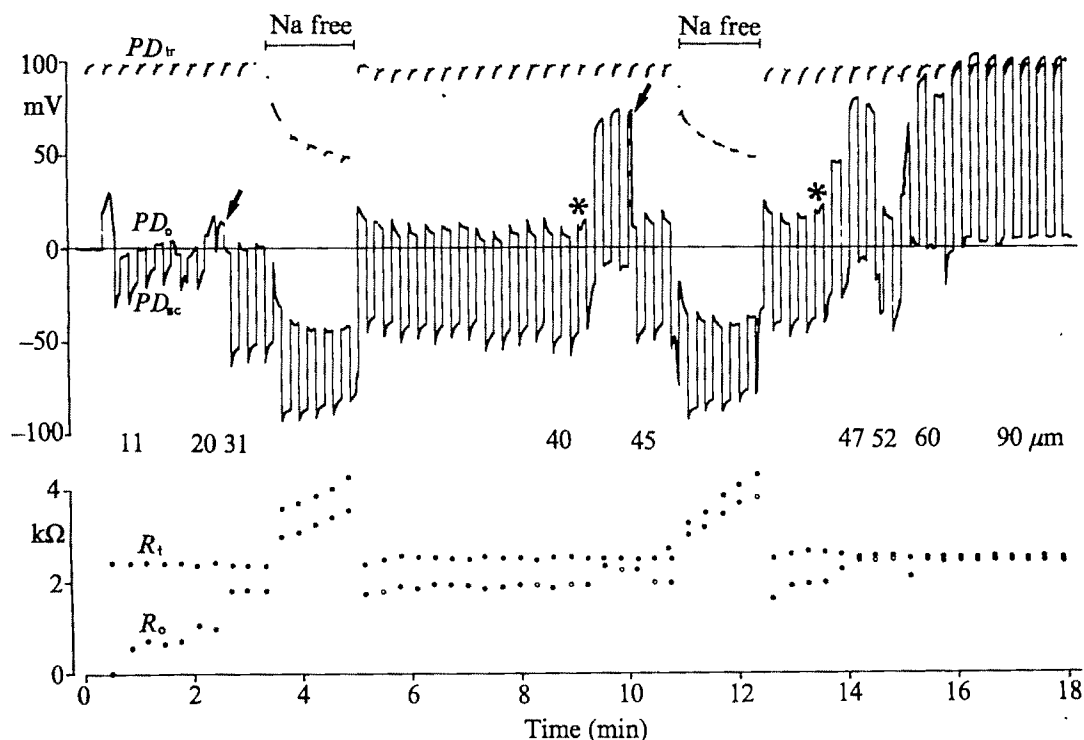


Fig. 1 Microelectrode impalement of the skin of *R. esculenta* (SO₄-Ringer) showing recording from two successive cell layers with intermediate location of the microelectrode in extracellular (corium facing) spaces. Access to intracellular spaces is labelled by solid arrows; indications of cell rupture (breakdown) are marked by asterisks. Potentials given are with respect to the epithelial bathing solution. Trans-epithelial potential (PD_{tr}) and potential difference across the outer border were recorded at the same time; PD_{sc} was recorded during short-circuiting the skin (automatically every 6 s for 6 s). The microelectrode position is given in μ m relative to the skin surface. R_i and R_o are calculated from the instantaneous changes of PD_{tr} and PD_o upon transepithelial injection (5 μ A). The skin surface was indented by the microelectrode. Thus, advancement would not be transferred into distance penetrated within the epithelium, but indicates maximal values of microelectrode movement.

Table 1 Mean values of PD_{sc}^* , PD_b^\dagger and R_o/R_t^\ddagger

a PD_{sc} (mV)	b cell 2 cell 1	a PD_b (mV)	b cell 2 cell 1	a R_o/R_t	b cell 2 cell 1
-64 ± 7	$1.031 \pm .060$	-97 ± 6	$0.980 \pm .031$	$0.758 \pm .035$	$1.060 \pm .022$

*Short-circuit intracellular potential.

†Potential difference across the basolateral membranes.

‡Fraction of the transepithelial resistance penetrated by the microelectrode.

Values observed in the first impaled cell layer are in columns *a*; columns *b* contain the ratios of these values to those recorded after intermediate extracellular location from the second cell layer. $n = 8$; \pm s.e.m.

larger amounts of Na exchanged across the junctional complexes and became evident as a second component.

These microelectrode observations which demonstrate that intercellular exchange of ions occurs in normal (unpoisoned and non-depolarised) frog skins are in accordance with previous suggestions from histochemical ATPase localisation⁶. The outermost reacting cell layer¹⁰ would then be the dominating location of active Na extrusion into the intercellular spaces but at least at high rates of influx, excess Na may pass into deeper cell layers to be transported out of this facultative transport pool.

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Incorporation of $^{32}\text{P}_i$ into pyruvate dehydrogenase phosphate in mitochondria from control and insulin-treated adipose tissue

PYRUVATE dehydrogenase complexes (EC 1.2.4.1) isolated from mammalian sources have been shown to be inactivated by phosphorylation of the α subunit of the pyruvate decarboxylase component. This covalent modification is brought about by a tightly bound, ATP-requiring kinase, which is activated by NADH and acetyl CoA and inhibited by pyruvate, Ca^{2+} , thiamine pyrophosphate and ADP. Reactivation is catalysed by a specific phosphatase which requires both Mg^{2+} and Ca^{2+} (refs 1–6). In rat epididymal fat pads, the proportion of the complex in the active non-phosphorylated form is increased following brief exposure of pads to insulin^{6–8} and this effect persists during preparation and subsequent incubation of mitochondria with oxidisable substrates such as oxoglutarate and malate^{9,10}. The mechanism by which the interaction of insulin with the cell membrane results in the changes of this mitochondrial enzyme system has not been established. It is possible that insulin may act through activation of the phosphatase by an increase in mitochondrial Ca^{2+} concentration and this possibility has been explored extensively in this laboratory^{3,9,11}. In contrast, other workers^{7,8} have argued that the effect of insulin is brought about by an inhibition of the

kinase caused by a lowering of the mitochondrial concentration ratio ATP:ADP. We report here evidence against the latter hypothesis.

In fat pad mitochondria incubated with oxoglutarate and malate in the presence of $^{32}\text{P}_i$, we have found that the only appreciably phosphorylated protein component within the mitochondria is the α subunit of pyruvate dehydrogenase. Moreover, since the incorporation of phosphate into this subunit is slow compared with the equilibration of mitochondrial ATP with the medium $^{32}\text{P}_i$, the rate of incorporation gives an estimate of the kinase activity within the intact mitochondria. No evidence was found for any decrease in kinase activity in fat pad mitochondria from insulin-treated tissue—indeed, its activity seemed to be increased.

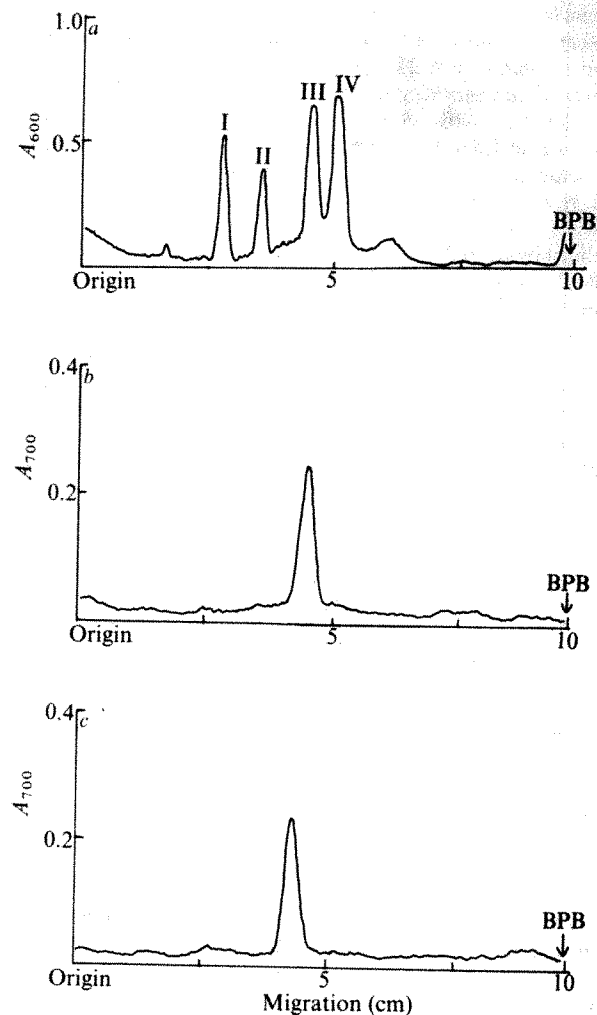


Fig. 1 SDS-polyacrylamide gel electrophoresis of components of *a, b*, pig heart ^{32}P -pyruvate dehydrogenase phosphate and *c*, of a perchloric acid precipitate of fat pad mitochondria following incubation with $^{32}\text{P}_i$ (0.2 mM and 3,000 c.p.m. μmol^{-1}) for 10 min. Samples were suspended in 50 μl of Bromophenol blue (BPB; 0.2 mg ml^{-1}), sodium dodecyl sulphate (SDS; 40 mg ml^{-1}), sucrose (0.2 g ml^{-1}), sodium phosphate (0.02 M), pH 7.0, and then incubated for 1.5 h at 60 °C. A portion (10 μl) was applied to a 7.5% (w/v) polyacrylamide gel (0.6 cm \times 10 cm) containing bisacrylamide (2.7 mg ml^{-1}), SDS (1 mg ml^{-1}) and 50 mM sodium phosphate, pH 7.0 (ref. 12). Electrophoresis was carried out at 20–25 °C for about 3 h at 8 mA per tube. After electrophoresis protein components were stained with Coomassie Brilliant Blue and the gels scanned at 600 nm (*a*). I, Dihydrolipoate transacetylase; II, dihydrolipoate dehydrogenase; III, pyruvate decarboxylase, α subunit; IV, pyruvate decarboxylase, β subunit. The ^{32}P -labelled components were located by autoradiography after the gels were washed in 7% acetic acid overnight, and the autoradiographs scanned at 700 nm (*b* and *c*). (For preparation of ^{32}P -pyruvate dehydrogenase phosphate, see ref. 3.)

Epididymal fat pads were obtained from fed male Wistar rats (180–220 g), incubated for 30 min in bicarbonate-buffered medium containing fructose (2 mg ml⁻¹) with or without insulin (10 munit ml⁻¹), and mitochondria prepared as previously described⁹. Mitochondria (about 2 mg protein ml⁻¹) were pre-incubated in air-equilibrated KCl (125 mM), EGTA (2 mM), Tris-HCl (25 mM) pH 7.4 containing 2-oxoglutarate (2 mM), malate (0.2 mM) and potassium phosphate (0.2 mM) at 30 °C for 3 min. As found previously¹⁰, the pyruvate dehydrogenase activity had by this time reached a steady-state value which remained constant for the next 10–15 min. In mitochondria from control tissue about 15% of the complex was in the active form and this was increased in mitochondria from insulin-treated tissue to about 25%. Addition of ³²P_i was made after the 3-min preincubation and incubation continued at 30 °C for varying times up to 20 min. For measurement of ³²P_i incorporation into the α subunit of pyruvate dehydrogenase, incubations were terminated by addition of perchloric acid (final concentration 2%). Insoluble proteins were precipitated by centrifugation (90 s at 10,000g in Eppendorf 3200 minifuge) and analysed by SDS-polyacrylamide electrophoresis as described in legends to Figs 1 and 2. Recovery of ³²P-labelled pig heart pyruvate dehydrogenase phosphate by this procedure was greater than 90%. For the measurement of ATP specific activity, mitochondria were separated from the ³²P_i-containing medium by centrifugation through an oil layer into a perchloric acid-glycerol mixture (see legend to Fig. 2 for details). Measurements of initial pyruvate dehydrogenase, total pyruvate dehydrogenase and glutamate dehydrogenase activities were made as described previously^{9,11} in samples of mitochondria incubated in identical conditions but without addition of ³²P-phosphate. For pyruvate dehydrogenase phosphate, a unit of enzyme activity is that amount which yields one unit of pyruvate dehydrogenase ($\mu\text{mol min}^{-1}$ at 30 °C) after complete conversion into the non-phosphorylated form with phosphatase in the presence of Mg²⁺ and Ca²⁺.

As expected, SDS-polyacrylamide electrophoresis of the proteins in fat pad mitochondria indicated the presence of a large number of different components. But after incubation of the mitochondria with ³²P_i in the presence of oxoglutarate and malate, only a single, symmetrical peak of radioactivity was separated by electrophoresis (Fig. 1c). The following evidence indicates strongly that this component is the α subunit of pyruvate dehydrogenase phosphate: (1) The *R_f* of the peak (0.45) was the same as the α subunit of purified pig heart ³²P-pyruvate dehydrogenase phosphate; little or no incorporation of ³²P was found corresponding to the other subunits of the complex (Fig. 1, a and b). Components of the pig heart complex and fat pad mitochondria have also been separated by SDS-polyacrylamide electrophoresis on adjacent tracks of a polyacrylamide slab. Incorporation of ³²P into protein components from fat pad mitochondria with *R_f* values corresponding to the β subunit (*R_f* 0.52), dihydrolipoate transacetylase (*R_f* 0.29) and dihydrolipoate dehydrogenase (*R_f* 0.36) was less than 3% of the incorporation into the peak which migrates as the α subunit (*R_f* 0.45) of pig heart pyruvate dehydrogenase. Similar results have been found using mitochondria from rat heart, liver and kidney incubated with succinate as substrate. (2) Maximum incorporation of ³²P into the peak was equivalent to 0.6–0.8 nmol phosphate per unit pyruvate dehydrogenase phosphate (Fig. 2). This level of incorporation is close to that observed with preparations of pyruvate dehydrogenase purified from pig heart³. (3) Treatment of phosphate buffer extracts of fat pad mitochondria with partially purified pig heart pyruvate dehydrogenase phosphatase¹¹ in the presence of Mg²⁺ and Ca²⁺ before precipitation of the proteins with perchloric acid resulted in complete disappearance of ³²P incorporation into the peak (*R_f* 0.45). (4) Incubation of fat pad mitochondria with pyruvate (2 mM) in the presence of oxoglutarate, malate and phosphate leads to a large decrease (by 70–80%) in the mitochondrial content of pyruvate dehydrogenase phosphate because pyruvate is a specific inhibitor of the kinase¹⁴. In these conditions, the

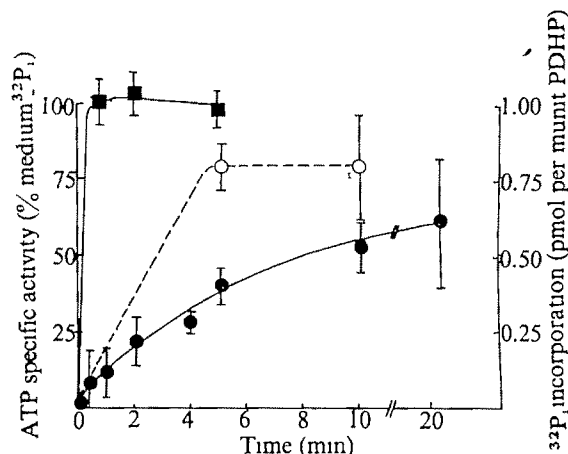


Fig. 2 Time courses of ³²P incorporation into the α subunit of pyruvate dehydrogenase phosphate (●, ○) and γ -³²P-ATP specific activity (■) in fat pad mitochondria incubated with ³²P_i. Mitochondria were prepared from fat pads incubated in the presence (---) or absence (—) of insulin, 10 mU ml⁻¹. After precipitation of the mitochondria in medium containing phosphate, oxoglutarate and malate for 3 min, ³²P_i was added to give 0.2 mM and a final specific activity of 1,000 to 5,000 c.p.m. pmol⁻¹ and incubation continued for various times (see text for details). After SDS-polyacrylamide electrophoresis as described in Fig. 1, the gels were cut into transverse slices (5 mm), dissolved in hydrogen peroxide (30% w/v) and then neutralised with 1 N HCl. Samples were taken up in a methoxy-ethanol-based scintillation fluid¹¹ for determination of radioactivity. In all cases a single peak of radioactivity with an *R_f* close to 0.45 was found (see Fig. 1c), incorporation into the α subunit was taken as the radioactivity in this peak with a correction (<5%) for background radioactivity taken as the mean of values in adjacent slices. Values are expressed as pmol of medium ³²P_i incorporated per munit of pyruvate dehydrogenase phosphate. Specific activity of intramitochondrial γ -³²P-ATP was measured by the method of England and Walsh¹³ modified for small concentrations of ATP (about 5 μ M). For these measurements, after incubation with ³²P_i for varying times, the mitochondria were centrifuged through an oil layer (silicone oil MSS60: dinonyl phthalate (3:2)) into perchloric acid (3%) containing glycerol (25% w/v). Each value is the mean \pm s.e.m. of observations made on three to eight separate mitochondrial preparations.

maximum incorporation of ³²P_i into the peak (*R_f* 0.45) was decreased in three separate preparations of mitochondria by a very similar percentage. Closely parallel decreases in ³²P_i incorporation and pyruvate dehydrogenase phosphate content were also apparent on incubating mitochondria in the presence of uncoupler (FCCP, 1 μ M).

The incorporation of ³²P_i into the α subunit took 10–15 min to reach completion in mitochondria from control tissues. In mitochondria from insulin-treated tissue, incorporation seemed complete by 5 min (Fig. 2). The maximum incorporation (0.6–0.8 nmol phosphate per unit pyruvate dehydrogenase phosphate) was not significantly affected by insulin treatment. The specific activity of mitochondrial ATP was found to rise to that of the medium phosphate within 30 s (Fig. 2). Consequently the rate of incorporation of ³²P_i into the α subunit gives an estimate of the rate of the kinase reaction within the mitochondria, using the present conditions where no changes in the phosphorylation state of pyruvate dehydrogenase complex are occurring. The rate was found to be significantly greater in mitochondria from insulin-treated tissues. The increase in incorporation after 5 min was $44 \pm 11\%$ if results were expressed in terms of total pyruvate dehydrogenase activity and $36 \pm 9\%$ if expressed in terms of mitochondrial glutamate dehydrogenase (mean \pm s.e.m. for seven separate paired preparations of mitochondria). In two experiments, measurements were also made after incubation for 2 min and again increases of incorporation (by 17 and 29%) were observed.

These results provide strong evidence against the hypothesis^{7,8} that the increase in the non-phosphorylated form of pyruvate dehydrogenase in adipose tissue exposed to insulin is brought

about by a decrease in kinase activity. Moreover, measurements of some of the known effectors of the kinase (ATP, ADP, NAD, NADH, acetyl CoA, CoA) have been made in mitochondria from control and insulin-treated tissue (refs 10, 15 and unpublished work with B. J. Bridges), but no clear evidence has been found for any changes which could result in a decrease in kinase activity. In particular, no reduction in the ATP:ADP concentration ratio has been observed. We conclude that it is more likely that the effect of insulin is mediated through an increase in the activity of the phosphatase. The subsequent increase in the active non-phosphorylated form of pyruvate dehydrogenase may then result in the enhanced rate of phosphorylation observed in the present studies. Since no differences in the activity of pyruvate dehydrogenase phosphatase persist in extracts of mitochondria from control and insulin-treated tissue¹⁶, insulin may act through changes in the mitochondrial concentration of one or more effectors of the phosphatase.

The only two effectors of the phosphatase that have been recognised to date are Mg^{2+} and Ca^{2+} which both activate the enzyme^{1,3,6} and so the strong possibility remains that the effects of insulin may be brought about by an increase in the free concentration within adipose tissue mitochondria of one or both of these divalent metal ions.

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Circular and elongated linear forms of measles virus nucleocapsid

PARAMYXOVIRUSES are assuming increasing importance as possible causative agents of several human diseases^{1,2}. To date little is known about their mode of replication, although it has been suggested that the nucleocapsid replicates without extensive dissociation of the protein from the nucleic acid component³. Previous reports on the structure of nucleocapsids extracted from virions or infected cells, with or without further purification, have shown by electron microscopy that the molecules are linear, and although short pieces can be found, the majority are about 1.0 μ m long⁴. We report here the occurrence of rare circular forms of measles nucleocapsid as well as linear molecules of greater than normal length.

Three strains of measles virus have been examined; a non-haemagglutinating strain⁵ (P9), a haemagglutinating strain (TC243) and measles virus isolated during a transfection experiment using DNA extracted from the brain of a multiple sclerosis patient (H.V.T. and Haire, unpublished). The origin of this virus (provisionally designated Miller virus), has not yet been established. The viruses were

grown in human embryonic lung (HeLu) and Vero cell monolayers which were infected with virus (about 1 plaque-forming unit per cell) by adsorption for 1 h at 37 °C and cultured in 199 medium + 10% calf serum at 37 °C for various times. Material for electron microscopy was prepared, with equivalent results, by lysing monolayers with 2% phosphotungstic acid or by rubbing off cells into the

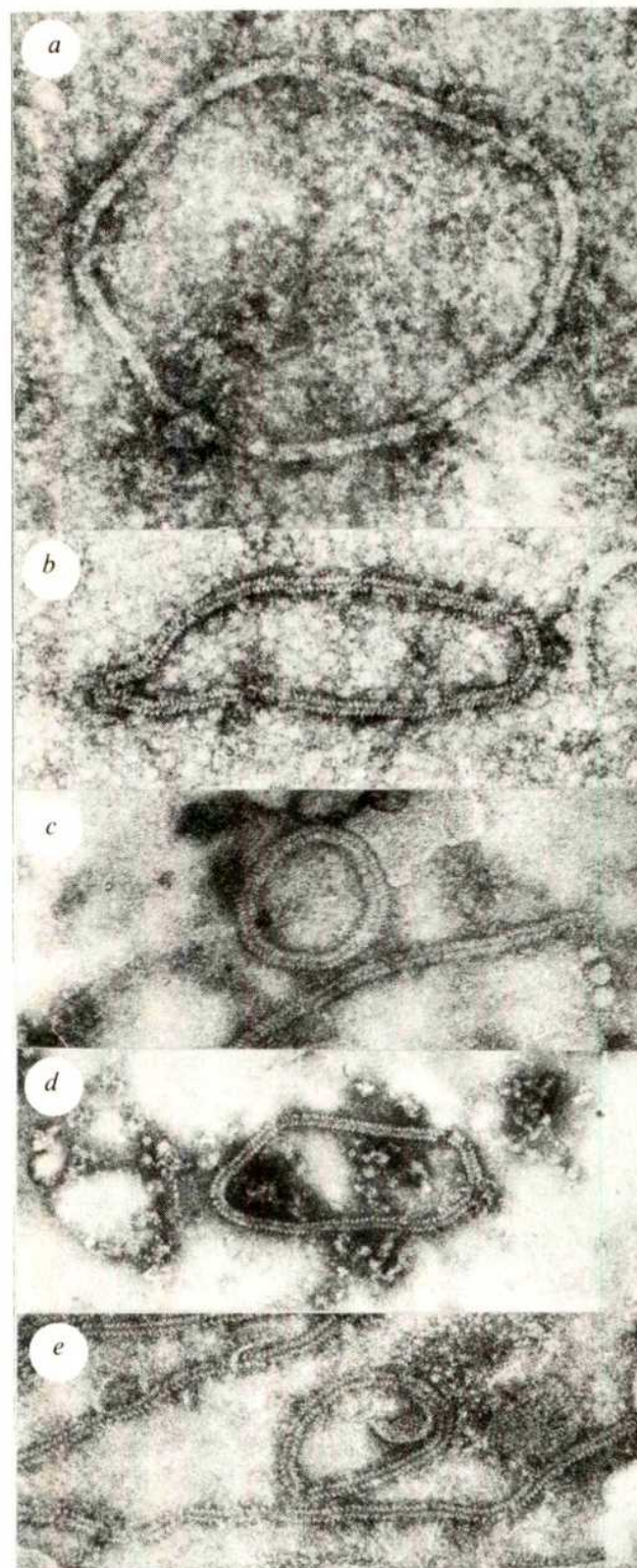


Fig. 1 Circular nucleocapsids of different sizes: a, b, and c, Miller virus grown in HeLu cells 1.5, 1.1 and 0.5 μ m respectively; d, TC243 virus grown in HeLu cells, 0.6 μ m; e, P9 virus grown in Vero cells, 0.5 μ m. $\times 125,000$.

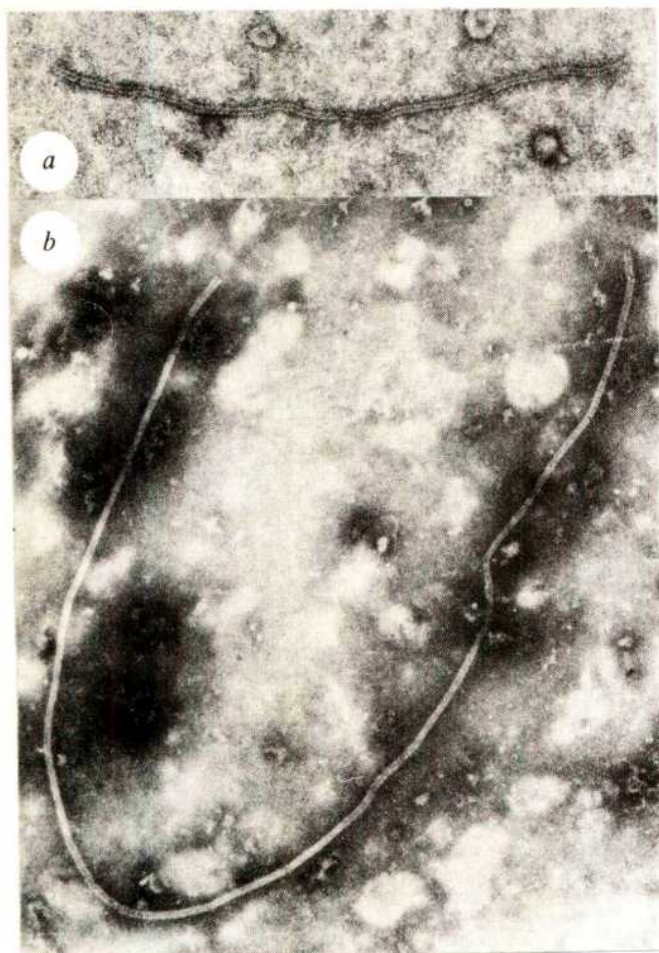


Fig. 2 Linear nucleocapsids of different lengths: *a*, Miller virus grown in HeLu cells, 1.2 μm ; *b*, P9 virus grown in HeLu cells, 3.6 μm . $\times 60,000$.

medium followed by centrifugation at 30,000 r.p.m. for 1 h with resuspension of the pellet in phosphate-buffered saline (PBS), or by washing monolayers with distilled water followed by Dounce homogenisation and low speed centrifugation to remove debris. The preparations, without further purification, were put on to carbon-Formvar coated grids, excess solution was removed with filter paper and, in the latter two methods, grids were then stained with 2% phosphotungstic acid. Grids were examined immediately in an AEI Corinth 500 electron microscope.

Unbroken circular nucleocapsids were found in all three virus strains and both cell cultures (Fig. 1), but were rare compared to the linear forms. The size of the circles varied between 0.2 and 1.5 μm and could be found in preparations from cultures from 18 h post-infection onwards, that is, at the normal time of nucleocapsid accumulation⁶. Present techniques do not indicate whether the number of circles varies during the growth cycle of the virus. In addition to unbroken molecules a small proportion of molecules showing one or more breaks or discontinuities were observed. It is not possible to ascertain whether these breaks are real or artefacts of preparation. No rings or linear molecules were found in uninfected cultures.

The most frequently found length of linear nucleocapsid was about 1.1 μm (Fig. 2*a*), but we also observed abnormally long nucleocapsids (Fig. 2*b*) in all three virus strains with one of nine times this length in Miller virus grown in HeLu cells. Long molecules seem to increase in number and length with time after infection, particularly in HeLu cells in which the cytopathic effect is less destructive

permitting observation for times up to 3 weeks post-infection. Although no circular molecules have been described previously for any paramyxovirus, rare nucleocapsid molecules of 2 and 4 μm lengths which were considered to be end-to-end aggregates of normal length nucleocapsids have been reported for the parainfluenza virus SV5 (ref. 2).

Our results do not allow any conclusions as to the origin and function of either the circular or the long molecules, but they may indicate, assuming that the molecules contain RNA, that there are circularisation processes for RNA; evidence for this has recently been presented in an oncornavirus system⁷. Since no ligase for RNA is known, we speculate that circles arise from the presence of complementary ends on some or all of the RNA molecules. The long molecules could result from end-to-end linkage of similar RNA molecules.

Although it cannot be ruled out that the circles are accidental forms of nucleocapsid with no role in the normal replication process, nevertheless, we suggest two possible functions for these molecules. First, that the circles represent the templates from a replicative intermediate arising during a "rolling circle" type of replication. Such a model could also provide an alternative explanation for the presence of long molecules. The varying size of the circles could be accounted for by the presence of replicating defective genomes. Second, the circles are encapsidated RNA molecules which function as templates for DNA replication (presumably by a reverse transcriptase) ultimately producing circular double-stranded DNA molecules which could be in suitable form for integration into the cell genome. This would provide a mechanism to explain the existence of infectious DNA molecules which have been obtained from cultures persistently infected with measles⁸. There may be a relationship between such a mechanism and the integration of virus-specific sequences which has been suggested to occur in diseases such as systemic lupus erythematosus and multiple sclerosis⁹.

It will be interesting to see whether similar forms of nucleocapsid can be identified in other RNA virus systems.

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RNA homology between subacute sclerosing panencephalitis and measles viruses

VIROLOGICAL and immunological studies in subacute sclerosing panencephalitis (SSPE) have associated this disease with measles virus^{1,2}. Measles-like virus (referred to as SSPE virus) has been isolated from brain material of patients with SSPE and all SSPE patients reveal an hyperimmune reaction to measles virus antigen. In spite of these major findings the aetiology and pathogenicity of SSPE is still not understood, and the rarity and rural prevalence of this disease

remain unexplained³. If measles virus is involved then additional factors either host or virus dependent, must determine the disease since the occurrence of measles virus and its acute infection cannot be correlated with SSPE. So far, no major host factor, either immunological or genetic, has been discovered which would answer these questions⁴.

Biological and ultrastructural comparisons of SSPE and measles viruses have indicated some differences⁵⁻⁷. The present communication compares several SSPE isolates with measles virus in terms of genome homology. The genome of measles and SSPE viruses consists of a single-stranded piece of RNA which has a sedimentation coefficient of 50S (refs 8 and 9). After infection this RNA is transcribed into complementary messenger (m)RNA molecules which sediment at approximately 18S in sucrose gradients. The genome homology of different viruses can be compared simply by hybridising either the mRNAs of different viruses to one radioactive labelled genome or vice versa. One problem with hybridisation experiments involving SSPE viruses is that in the early passage levels after isolation, large amounts of defective genomes are produced. These RNAs contain some of the sequences of the infectious genome and have the same sedimentation coefficients as the virus messengers^{10,11}. Thus, if "18S" RNAs from infected cells are used in hybridisation experiments they will "self-hybridise". Such a background of self-hybridisation would give erroneous results in any hybridisation experiments. The mRNAs were therefore purified on the basis of their poly(A) content by chromatography on oligo d(T)-cellulose. A second problem is the difficulty in producing a complete set of highly labelled mRNAs or genome RNAs because of the long and variable growth rates of these viruses. Therefore we have used a competition-hybridisation method where only one or two RNAs need to be labelled and the different growth rates are unimportant.

The viruses compared in the present study were two measles viruses (the attenuated Edmonston strain and Woodfolk, a wild measles strain), three strains of SSPE (Mantooth, Lec and Jac), the immunologically related Onderstepoort strain of canine distemper virus (CDV) and a bovine-meningo encephalitis virus (107 virus). The latter virus has been isolated from the brain of a calf which had died from a meningo-encephalomyelitis, and on the basis of its morphological and biological characteristics belongs to the measles-CDV-Rinderpest virus group¹². The RNA of parainfluenza 1 6/94 virus and Vero cell ribosomal RNA were used as controls.

All viruses were grown in Vero cells except 107 virus which was cell associated with BHK cells, and mRNAs were isolated between 18 and 60 h after infection. In the competition hybridisation experiments we selected two viruses: the Edmonston and Mantooth strains as proto-types of measles and SSPE respectively. Unlabelled genome RNA was prepared from purified nucleocapsids as previously described⁹. The corresponding mRNAs of these two viruses were labelled with ³H-uridine by adding large quantities (100 μ Ci ml⁻¹) of isotope to actinomycin D-treated infected cells. The mRNAs were extracted and purified by sucrose-gradient sedimentation and oligo d(T)-cellulose chromatography (Fig. 1). Unlabelled mRNAs of all the viruses were then purified from infected cells using the same method. The genetic relatedness or comparative homology of the viruses could be measured by the ability of the unlabelled or "cold" mRNAs to compete with the hybridisation of the labelled mRNA to its corresponding genome.

A typical set of results obtained is shown in Figs 2 and 3. Figure 2 shows the competition profiles of the mRNAs against the hybridisation of ³H-labelled Edmonston mRNA to its genome. It can be seen that the Woodfolk strain of measles virus shares 100% homology with the Edmonston. Similarly, the three SSPE viruses tested shared 100% homology with Edmonston measles. CDV and 107 virus shared

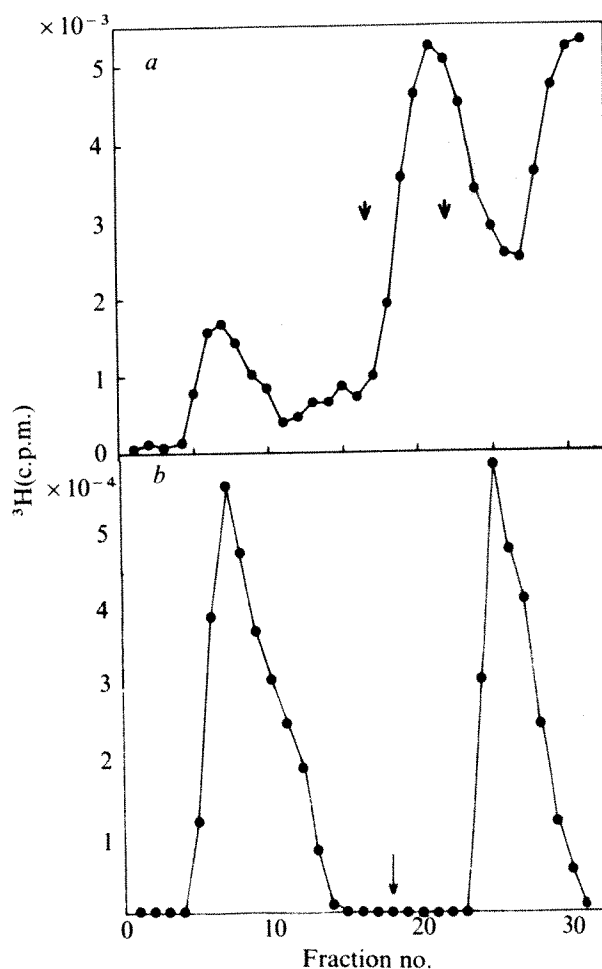


Fig. 1 Purification of virus-specific mRNAs. RNA was extracted from infected cells by the following procedure. Cells were washed twice with ice-cold NTE buffer (0.01 M Tris-HCl (pH 7.5), 0.1 M NaCl, 0.001 M EDTA), scraped into fresh NTE buffer and collected by centrifugation at 2,000g for 10 min at 4 °C. Pellets were resuspended in sterilised distilled water (0.1 ml) and allowed to swell on ice for 15 min. Cells were disrupted by douncing 10 strokes in a tight-fitting glass homogeniser, and nuclei were removed by centrifugation at 800g for 8 min. Supernatants were removed, made 2% in sodium dodecyl sulphate (SDS) and heated at 56 °C for 2 min. Released RNA was directly sedimented on 35 ml linear 15 to 30% (w/w) sucrose gradients in NTE buffer containing 0.5% (w/v) SDS. Gradients were centrifuged for 16 h at 20,000g at 20 °C. After centrifugation, fractions were collected from the bottom of the tube using an LKB pump and an ISCO fractionator which measured absorbance continuously at 254 nm. Aliquots (25 μ l) of each fraction were precipitated with 3 volumes of 10% (w/v) trichloroacetic acid (TCA). Precipitates were collected using a Millipore Manifold 3025 filtration apparatus, and radioactivity was counted in Bray's solution. A typical radioactivity profile for Edmonston-measles virus is shown in *a*. RNA which sedimented at 12 to 36S (fractions 25-14) was pooled and precipitated overnight in 2 volumes of ethanol at -20 °C. Precipitates were collected by centrifugation at 8,000g for 30 min and allowed to dry at room temperature for 20 min. Samples were dissolved in TE buffer (0.01 M Tris-HCl (pH 7.4), 1.5 M LiCl, 0.001 M EDTA), allowed to stand at 4 °C for 24 h and then centrifuged at 12,000g for 25 min. The pellets which contained double-stranded RNA intermediates were discarded and the supernatants (predominantly single-stranded RNA) were precipitated in 2 volumes of ethanol as described above. Precipitates were collected, dissolved in 0.01 M Tris-HCl (pH 7.5), 0.5 M NaCl, 0.001 M EDTA containing 0.1% (w/v) SDS (binding buffer) and applied to 0.4 g of oligo d(T)-cellulose in a disposable pipette column. The column was washed with 8 ml of binding buffer and bound RNA was eluted with 0.005 M Tris-HCl (pH 7.4), 0.001 M EDTA containing 0.1% (w/v) SDS. Radioactivity levels were determined (Fig. 1*b*) and the purified mRNA precipitated with ethanol. The arrows in *a* represent the sedimentation position of Vero cell 28S and 18S ribosomal RNA; in *b* the arrow indicates the change of buffer from binding to elution.

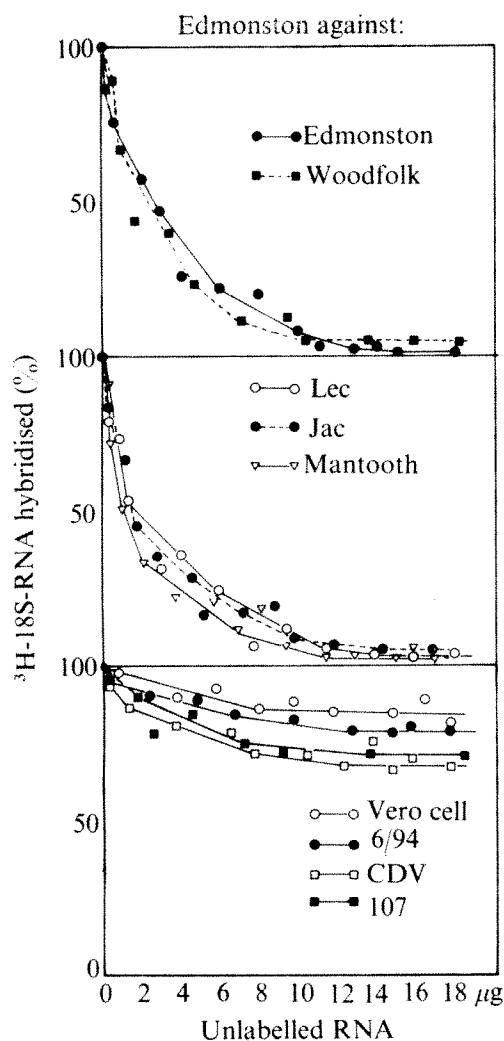


Fig. 2 Competition of unlabelled mRNAs on the hybridisation of Edmonston virus genome to its mRNA. Samples of Edmonston 50S RNA, ^3H -uridine labelled (12,000–14,000 c.p.m.) Edmonston mRNA and various amounts of unlabelled mRNAs were mixed in $2\times\text{SSC}$ ($\text{SSC} = 0.15\text{ M}$ sodium citrate, 0.15 M NaCl) containing 0.05% (w/v) SDS. Mixtures (final vol. $150\text{ }\mu\text{l}$) were added to glass tubes ($0.2\times 6.0\text{ cm}$). The tubes were briefly centrifuged to allow complete mixing of the RNAs and then sealed. After denaturation by boiling for 4 min hybridisation was allowed to occur by incubation at 60°C for 12–14 h. Tubes were cooled on ice, opened and the contents treated with ribonucleases (pancreatic $50\text{ }\mu\text{g ml}^{-1}$, T_1 $8\text{ }\mu\text{g ml}^{-1}$) at 37°C for 30 min. The reaction was stopped by the addition of 3 volumes of 10% (w/v) TCA and acid-insoluble radioactivity determined.

30% homology with measles virus, whereas parainfluenza 1 6/94 virus mRNA and Vero cell ribosomal RNA provided only a low and inconsistent level of competition. Figure 3 shows an identical experiment where the viruses were compared with the Mantooth strain of SSPE virus. It can be seen that all three strains of SSPE virus share 100% homology. However, in sharp contrast to the previous data the two measles virus strains were able only to compete out approximately 90% of the labelled SSPE mRNAs. It would therefore appear that, whereas all the genetic information contained in the measles genome is present in SSPE virus, the latter contains an additional piece of genetic information. CDV and 107 virus shared 38% homology with Mantooth SSPE, an increase in genetic relatedness to that observed with Edmonston measles virus. The parainfluenza 1 6/94 and Vero cell RNAs showed again only a low and inconsistent degree of relatedness. A summary of the percentage homologies of the different viruses to the measles and SSPE prototypes is shown in Table 1.

Table 1 Summary of competition-hybridisation results*

To:	% Similarity of	
	Edmonston	Mantooth
Edmonston	100	91
Woodfolk	97	88
Mantooth	99	100
Lec	98	102
Jac	99	99
CDV	29	43
107 Virus	28	41
6/94	19	18
Vero cell RNA	16	12

*The values represent the means of three individual experiments.

These results show that, whereas all the genetic information of measles virus is contained in SSPE viruses, the latter apparently contain an additional 10% information.

These results are in disagreement with the direct hybridisation experiments of Yeh⁸ who reported that the Woodfolk strain of measles virus contained only 60% of the genetic information of the SSPE-Lec virus genome. But in this work purified mRNAs were not used, saturation levels of annealing were not obtained, and the genetic relationship of the SSPE to the measles genome was not described.

An intriguing explanation for the results obtained would be that the viruses of SSPE may arise as a recombination

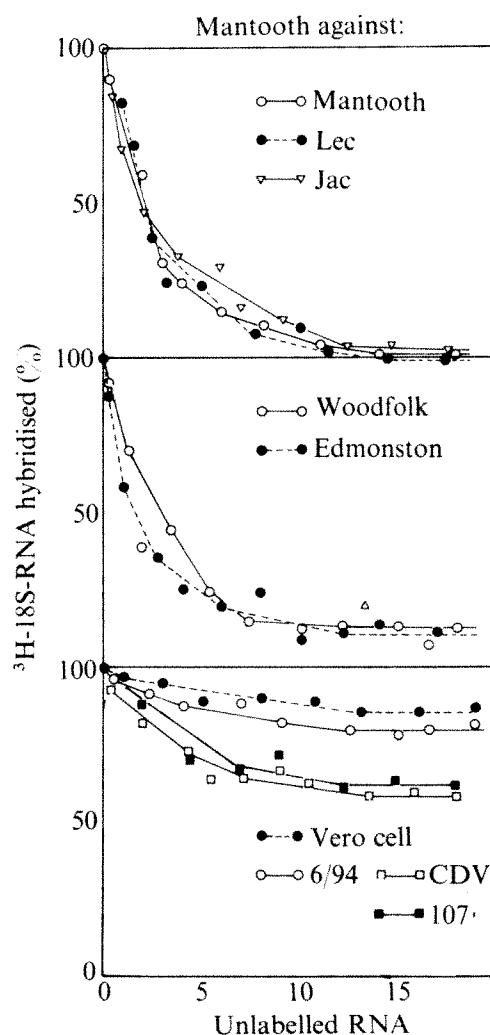


Fig. 3 Competition of unlabelled mRNAs to the hybridisation of Mantooth virus genome to its mRNA. Details were as described in Fig. 2. Each sample contained 16,000 c.p.m. of ^3H -uridine-labelled Mantooth mRNA.

of measles virus with another as yet unknown second virus. The additional information represents a molecular weight in the order of $4-5 \times 10^5$ which would have a sedimentation of about 12S. Defective RNAs of this size have been reported for paramyxoviruses including measles^{11,14}. Therefore it would seem likely that if a recombination occurs then it is with the intact genome of measles virus and a defective genome of the second virus.

The data exclude the possibility that the genomes of the SSPE viruses tested in the present study have arisen from a recombination between a complete and defective measles RNA genome, since the mRNA corresponding to the additional genomic information of SSPE is not present in measles-infected cells. The formation of covalently linked recombinant genomes is neither genetically nor biologically impossible. Recent work with vesicular stomatitis virus (VSV) has shown that mixed infections with defective and infectious viruses can result in the production of genetically stable recombinant genomes¹⁴ and a state of virus persistence *in vitro*¹⁵. A similar mechanism of mixed infection would account for the slow process of infection and the accompanying persistence observed in SSPE.

If such a double infection/recombination does occur in SSPE, it would account for two important features of the disease. Firstly, the rarity of the disease would be consistent with the low possibility of a mixed infection resulting in genetic recombination. Secondly, if the second virus is an animal RNA virus then it would account for the predominant rural distribution of the disease.

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Interferon-mediated protein kinase and low-molecular-weight inhibitor of protein synthesis

PROTEIN synthesis in cell-free systems from mouse L-cells pretreated with the antiviral agent interferon¹ shows an enhanced sensitivity to inhibition by double-stranded RNA (dsRNA)^{2,3}. The possible significance of this with respect to events in intact interferon-treated virus-infected cells has already been discussed^{2,4}. We have already shown that

the addition of small amounts of a post-ribosomal supernatant fraction from interferon-treated cells (interferon cell sap) renders protein synthesis in control cell-free systems sensitive to inhibition by dsRNA⁴. Our results are in accord with a two-step model involving an initial 100-fold activation of an inhibitor on incubation of the interferon cell sap with ATP and dsRNA (activation step), the activated inhibitor then interacting with the protein-synthetic system to inhibit translation (inhibitory step)⁵. In view of the dependence of the inhibition of protein synthesis upon incubation with ATP as well as dsRNA⁵ it was of interest to determine if a protein kinase(s) is involved, as seems to be the case in reticulocyte lysates in which phosphorylation of the initiator Met-tRNA binding factor (IF-E2) has been implicated following incubation of the lysates under a variety of inhibitory conditions including the presence of dsRNA (P. Farrell,

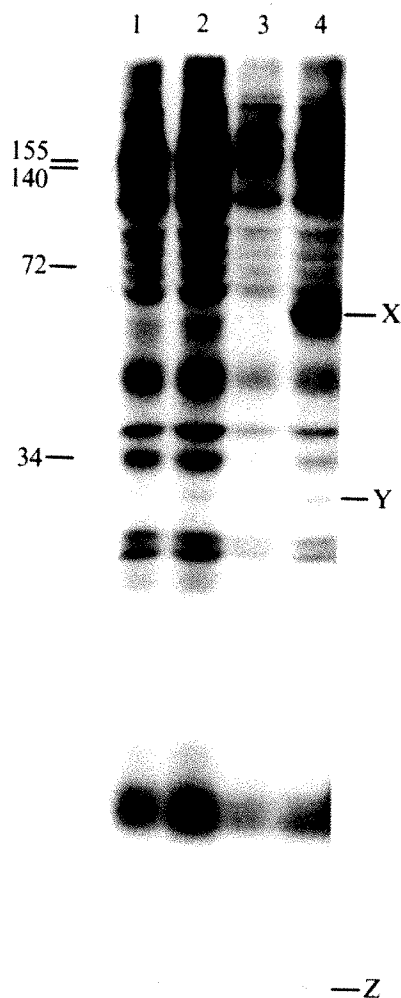
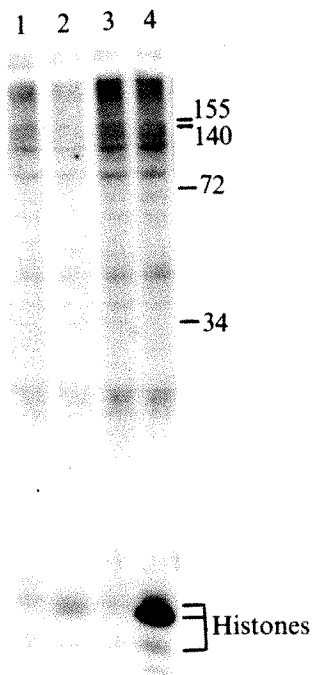


Fig. 1 A dsRNA-dependent protein kinase activity in cell sap from interferon-treated cells: electrophoretic analysis of phosphorylated polypeptides. Control cell sap minus (1) and plus (2) dsRNA. Interferon cell sap minus (3) and plus (4) dsRNA. Each cell sap (10 μ l) was incubated with 0.1 mM γ -³²P-ATP (8 Ci mmol⁻¹, Radiochemical Centre) plus or minus *Penicillium chrysogenum* phage dsRNA⁷ (400 ng ml⁻¹) in a final volume of 13 μ l. Incubation was for 40 min at 30 °C. Material equivalent to 5 μ l of each incubation was analysed by electrophoresis on a 10% polyacrylamide slab gel containing 0.1% sodium dodecyl sulphate (SDS)^{8,9}. An autoradiograph of the stained, dried gel is shown. The numbers to the left give the molecular weights in thousands of the major reovirus polypeptides λ 1, λ 2, μ 2 and σ 3¹⁰, included as markers. Interferon treatment of mouse L-cells with low doses (5 to 20 effective units ml⁻¹) of highly purified mouse interferon ($\geq 5 \times 10^7$ reference units mg⁻¹ protein) and the preparation of cell extracts and of the dialysed interferon and control cell saps were as described previously^{2,4}.



K. Balkow, T. Hunt and R. J. Jackson, personal communication⁶. In accord with this, we report here the presence of a dsRNA-dependent protein kinase activity in interferon cell sap. The inhibitor of protein synthesis which is activated on incubation of interferon cell sap with ATP

Fig. 2 Phosphorylation of histones by a dsRNA-dependent protein kinase in cell sap from interferon-treated cells: electrophoretic analysis of the phosphorylated histones. Control and interferon cell saps were first activated as in Fig. 1 but with unlabelled ATP (1 mM). Histone was then labelled by subsequent incubation of either the control cell sap (2 μ l) activated minus (1) or plus (2) dsRNA, or the interferon cell sap (2 μ l) activated minus (3) or plus (4) dsRNA, with 0.12 mM γ -³²P-ATP (8 Ci mmol⁻¹) and 1 μ l of 2 mg ml⁻¹ calf thymus histone (type IIA, Sigma). Incubation was for 30 min at 30 °C in a total volume of 5 μ l. Material equivalent to one fourth of each incubation was analysed by electrophoresis as in Fig. 1. An autoradiograph of the stained, dried gel is shown. The molecular weight markers were as in Fig. 1.

and dsRNA, however, is heat stable and of relatively low molecular weight. It does not correspond either to the major polypeptide product of the interferon-dsRNA specific phosphorylation, or, presumably, to phosphorylated IF-E2.

To determine whether a protein kinase might be involved in the activation of the inhibitor formed in response to incubation of the interferon cell sap with dsRNA and ATP⁵, the incubation was carried out in the presence of γ -³²P-ATP and the phosphorylated polypeptides were analysed by electrophoresis on polyacrylamide gels (Fig. 1). With interferon cell sap plus dsRNA (Fig. 1, channel 4) a major phosphorylated band (X) with an apparent molecular weight of about 60,000 (60K) and an additional band (Z), which migrated near the ion front, were observed which were not detected in corresponding controls. The phosphorylated polypeptide Y of approximately 30,000 (30K) in molecular weight was enhanced in the presence of dsRNA in both interferon and control preparations, but the increase was routinely greater in the interferon than the control (Fig. 1, channels 2 and 4).

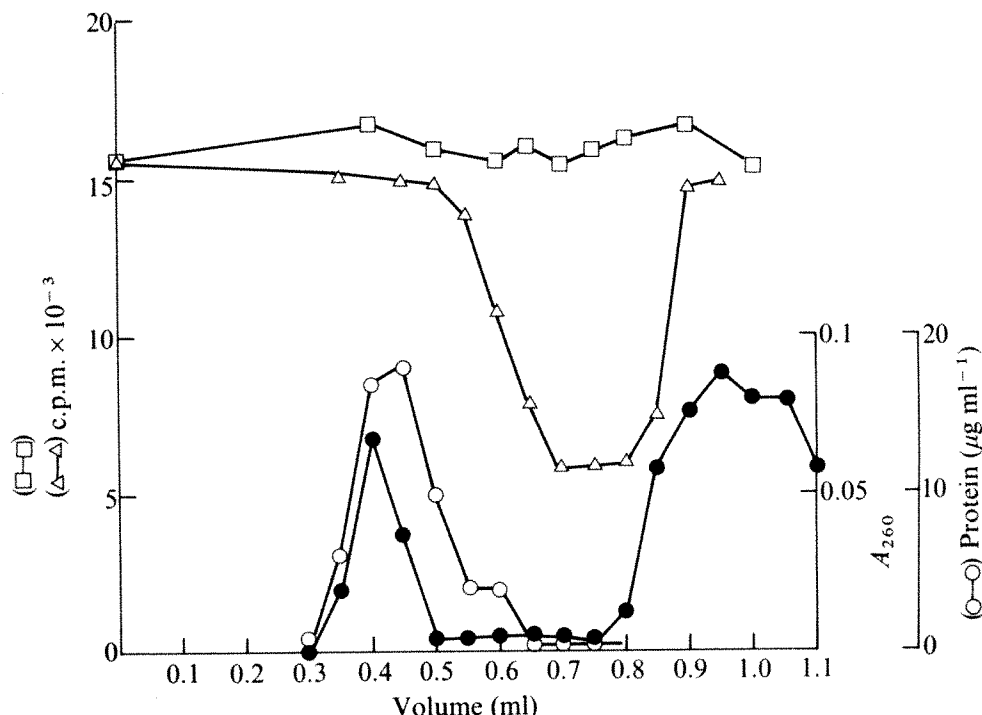


Fig. 3 Gel filtration chromatography of the activated inhibitor. Control and interferon cell saps were activated as in Fig. 1 but in the presence of unlabelled ATP (1 mM), heated to 90 °C for 5 min and centrifuged at 10,000 g for 10 min to remove denatured protein. Each supernatant (25 μ l) was fractionated by passage through a column (3 mm in diameter and 130 mm long) of Sephadex G-25 (Fine) equilibrated and eluted with 50 mM KCl and 20 mM Tris-hydrochloride (pH 7.6). Fractions (50 μ l) were collected and 2 μ l samples assayed for inhibitor activity on the translation of encephalomyocarditis virus (EMC) RNA in cell-free systems (25 μ l final volume) from control mouse L-cells^{4,5,9}. The left hand ordinate gives the incorporation (c.p.m. per 10 μ l sample) of a ¹⁴C-amino acid mixture into hot trichloroacetic acid-insoluble polypeptide in response to EMC RNA in the cell-free system^{4,5,9}. Interferon (△) and control (□) cell saps each activated in the presence of dsRNA. Interferon and control cell saps activated in the absence of dsRNA gave results essentially identical to the control plus dsRNA (□). The right hand ordinates give the protein concentration¹² (○) and absorbance at 260 nm (●) of the fractions obtained with the interferon cell sap which had been activated in the presence of dsRNA. The peak of optical density at 260 nm eluting between 0.8 and 1.1 ml represents primarily the ATP added for activation.

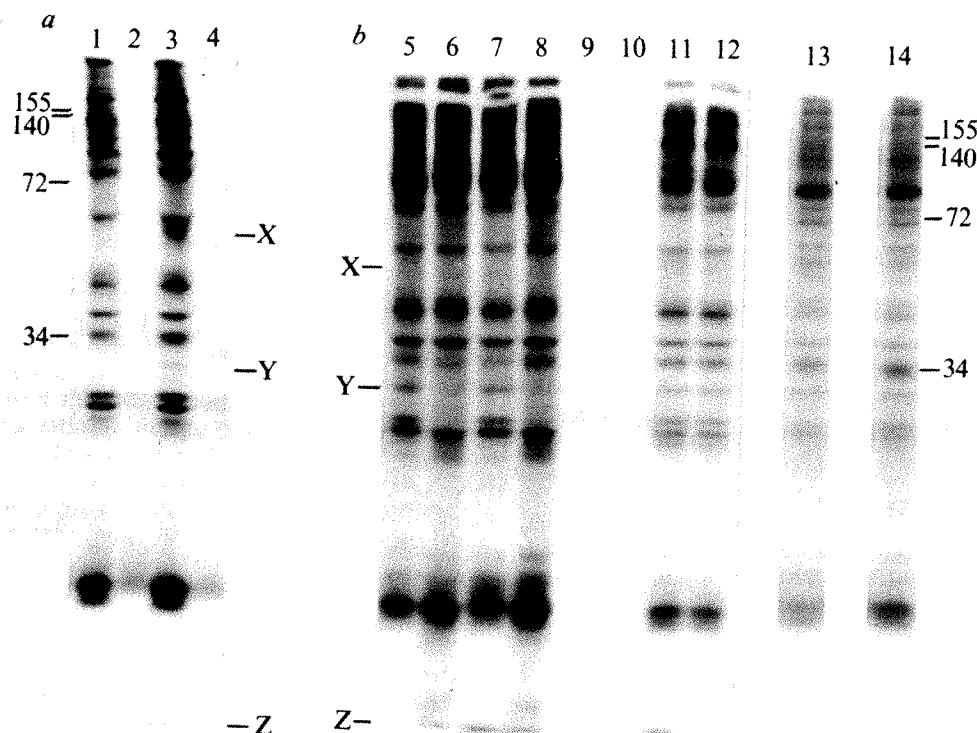


Fig. 4 *a*, Absence of phosphorylated polypeptides X and Y (Fig. 1) in the heat-stable inhibitor fraction. Interferon and control cell saps were incubated with γ - 32 P-ATP and dsRNA as in Fig. 1. A portion of each was heated to 90 °C for 5 min and centrifuged at 10,000g for 10 min. Samples (2 μ l) of the supernatant and of the original (unheated) activated material were analysed by electrophoresis on a polyacrylamide slab gel as in Fig. 1. Activated control cell sap (1) and supernatant after heating (2). Activated interferon cell sap (3) and supernatant after heating (4). *b*, Assay for protein kinase activity with activated inhibitor in the presence of histone, cell sap (S100), or post-mitochondrial supernatant (S10) fractions. Control and interferon cell saps were activated in the presence of dsRNA as in Fig. 1 but with unlabelled ATP (1 mM), heated to 90 °C for 5 min and centrifuged at 10,000g for 10 min. Samples (2 μ l) of each supernatant (interferon and control) were then incubated with γ - 32 P-ATP (2 μ l, 5 μ Ci μ l $^{-1}$, 8 Ci mmol $^{-1}$) in the presence of: (9 and 10) histone alone (1 μ l, 2 mg ml $^{-1}$ as in Fig. 2); (5 and 7) control cell sap alone (3 μ l); (6 and 8) control cell sap (3 μ l) plus histone (1 μ l); (11 and 12) interferon cell sap alone (3 μ l); (13 and 14) a post-mitochondrial supernatant (S10) fraction (3 μ l) from mouse L-cells which had been preincubated and dialysed in preparation for its use in the cell-free system 1,9 . Incubations for channels 7, 8, 10, 12 and 14 were with the supernatants from heated activated interferon cell sap, those for channels 5, 6, 9, 11 and 13 were with corresponding control material. Incubations were for 30 min at 30 °C. Samples representing one fourth of each incubation were analysed by electrophoresis on polyacrylamide slab gels 8,9 . Autoradiographs of the stained, dried gels are presented. The molecular weight markers were as in Fig. 1.

The appearance of these phosphorylated bands (Fig. 1, X, Y and Z) could reflect the presence of a new or increased kinase(s) or of new substrates (or both). As a test for an enhanced protein kinase activity, a potential substrate (calf thymus histone) was added to the interferon and control cell saps after activation and its phosphorylation examined by gel electrophoresis (Fig. 2). The marked increase, in the presence of interferon cell sap and dsRNA, in the phosphorylation of one of the major histone bands in particular (Fig. 2, channel 4) strongly suggests the activation of a specific kinase, although by no means excluding an additional or altered substrate as part of the explanation.

The major product of the interferon-dsRNA mediated kinase activity (X, Fig. 1) does not, however, appear to correspond to the inhibitor of protein synthesis activated on incubation of the interferon cell sap with ATP and dsRNA. This activated inhibitor is heat stable and seems to be of relatively low molecular weight. Thus after activation of the inhibitor by incubation of the interferon cell sap with dsRNA and ATP, heating to 90 °C for 5 to 15 min and centrifugation to remove the heat-denatured protein, the activated inhibitor is recovered *in toto* in the supernatant fraction. On passage of this supernatant through a column of Sephadex G-25 the inhibitor is retained separate from the peak of residual protein excluded from the column (Fig. 3). It seems unlikely,

therefore, that the activated inhibitor corresponds to either X or Y (Fig. 1). In fact, neither of these remain in the active supernatant on centrifugation after heating to 90 °C as above (Fig. 4, channels 1-4). (They can be recovered from the pellet, data not shown.)

Accepting that the activated inhibitor is neither X nor Y (Fig. 1), it was of interest to ask if a protein kinase is involved in the action of the activated inhibitor (inhibitory step). The heated activated inhibitor has no detectable kinase activity when assayed with histone as a substrate in the absence of added cell sap (Fig. 4, channels 9 and 10). Nor does it seem to function as an activator of protein kinase activity in cell sap or in the complete protein-synthetic system (Fig. 4, channels 5-8 and 11-14), although in the latter case a small quantitative difference in the 60K and 30K regions of the gel cannot be excluded (Fig. 4, channels 13 and 14). The differences in intensity of band Z here and in Fig. 1 are intriguing but variable and their significance is not yet clear.

There is, therefore, in cell sap from interferon-treated cells a dsRNA-dependent protein kinase(s) absent (or very much reduced) in corresponding controls. The main high-molecular-weight phosphorylated polypeptide products of this kinase(s) (X and Y, Fig. 1) do not, however, correspond to the heat-stable, low-molecular-weight inhibitor formed in response to incubation of interferon cell sap with ATP and dsRNA. Nor does the inhibition mediated by this

inhibitor seem to involve phosphorylation of these polypeptides. On the other hand, on a molecular weight basis alone, it is possible that a component of the band Z (Fig. 1) might be the active inhibitor, although our initial attempts to establish this have proved inconclusive. Certainly, until the biochemical nature of Z and of the activated inhibitor is known, the details of the activation and inhibitory steps worked out and the basis of the requirement for ATP understood, it would be premature to exclude a possible role for a protein kinase(s) in the inhibition of protein synthesis in these systems.

In more general terms, modulation of protein kinase activity by interferon may be of wider significance and the phosphorylation of histone (Fig. 2) could be of interest in this respect. It is conceivable that such kinases could be involved in the control of transcription or of other viral or cellular functions¹³ and thus account, in part at least, for the diversity of effects attributed to interferon.

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Primary and secondary variants in immunoglobulin heavy chain production

WE have isolated numerous variants of a mouse myeloma cell line which synthesised altered heavy immunoglobulin chains and normal light chains after treating the line with the acridine mustard ICR-191 (refs 1-4). The four types of primary variants that we found are shown in Table 1. The primary variant clones were generally stable but we did find one clone which on recloning produced subclones synthesising a variant heavy chain with molecular weight lower than that synthesised by the primary clone. This observation raised the question of whether certain primary variants might have a built-in instability and that the original mutation might be affecting a regulatory process. We were thus led to seek additional examples of spontaneous secondary variant production to see if this were a more general phenomenon. Here we report our results.

In the earlier experiments we isolated many independent

clones which fell into the four primary variant types. Two types synthesised heavy chains which are shorter than the parent and which lack the parental subclass serotype; two types synthesise heavy chains of a different subclass serotype from the parent. We also characterised the variants by the manner in which they assemble their heavy and light chains and by peptide analysis¹⁻⁴. Occasionally a primary variant clone showed decreased heavy chain synthesis while continuing to make the altered heavy chain. To retrieve those cells continuing to make the altered heavy chain, we recloned the primary variant cells. As expected, most subclones were of either the primary variant heavy chain type or the light chain producer type. The exception was the variant mentioned above synthesising a heavy chain of molecular weight 50,000: on recloning it we found, in addition to clones synthesising heavy chains of molecular weight 50,000 and clones that had lost the variant heavy chain synthesis completely, numerous subclones synthesising a variant heavy chain of molecular weight 40,000 (ref. 3).

In the present experiments the parental cell lines 45.6, 45.6.2.4 and 45.6.3.2 were derived from the MPC 11 tumour which synthesises an IgG-2b immunoglobulin⁵. The original variants were isolated from the parental population after mutagenesis with ICR-191^{3,4}. Mutagenised cells were cloned in soft agar and overlaid with an antiserum directed against either the completely reduced and alkylated MPC 11 (γ 2b) heavy chain or the Fc region (C-terminal half of the MPC 11 heavy chain). An antibody-antigen precipitate developed over those clones synthesising and secreting parental heavy chains. "Unstained" variant clones were retrieved from the agar and grown to mass culture. Unstained clones included clones which had ceased heavy chain synthesis completely, clones which continued heavy chain synthesis but had difficulty in secreting the heavy chain, and clones which synthesised an altered heavy chain not recognised by the overlay antiserum. The details of cell culture and cloning and the analysis of variant clones have been described previously^{3,4}.

To select secondary variants from individual primary variants synthesising altered heavy chains, we followed the same cloning-antibody overlay procedure without subjecting the cells to any additional mutagenesis. In this type of selection, the primary variant clones were "unstained" (as originally selected). "Stained" clones included those clones which secreted the primary variant heavy chain in sufficient quantity to yield a visible precipitate and those clones which synthesised and secreted a new immunoglobulin heavy chain which was recognised by the overlay antiserum. The same protocol would have selected clones synthesising and secreting the parental MPC 11 immunoglobulin, that is, revertants. To date, in several independent experiments, we have found no revertants.

Secondary variants have however been found in several experiments. In one study, a primary variant (45.6.3.2 ICR 11.19)^{3,6} which synthesised a heavy chain of molecular weight 50,000, was recloned and overlaid with an antiserum directed against the completely reduced and alkylated heavy chain of the MPC 11 immunoglobulin (IgG-2b). The primary variant had been selected originally as "unstained" by this same method. When the variant was recloned, sixteen of 478 clones were now found to be stained. Seven of these sixteen clones were grown to mass culture and analysed by Ouchterlony analysis of cytoplasmic contents and secreted immunoglobulin, and by electrophoresis of radiolabelled immunoglobulin on acrylamide gels containing sodium dodecyl sulphate. Five of the seven were identical to the primary variant (45.6.3.2 ICR 11.19) in that they synthesised heavy chains of molecular weight 50,000 which assembled with light chains to yield HL, L2 and L components. Now, however, they secreted the variant heavy chain in sufficient quantity to be detected as stained in the overlay procedure. The remaining two clones were found to synthesise heavy

Table 1 Size and serological characteristics of the heavy chains synthesised by variant types of the MPC cell line

Parent Variants	MPC 11	Size of heavy chain	Subclass	Anti:	Cytoplasm*		$\gamma 2a\%$	Anti:	Secretion*		$\gamma 2a\%$
					H†	Fc†			H†	Fc†	
Parent Variants	MPC 11	55,000	$\gamma 2b$		+	+	—		+	+	—
		(1) 50,000	neg		+	—	—		—	—	—
		(2) 40,000	neg		+	—	—		+	—	—
		$\gamma 2a$ serotype			+	+	+		—	—	—
		(3) 75,000	$\gamma 2a$		+	+	+		+	+	+
		(4) 55,000	$\gamma 2a$		+	+	+		+	+	+

*Ouchterlony analysis as in ref. 3.

†Anti-H = rabbit anti-MPC 11 heavy chains

‡Anti-Fc = rabbit anti-MPC 11 Fc

§Anti- $\gamma 2a$ = rabbit anti-LPC1 (IgG-2a κ).

chains of increased (normal) size (molecular weight 55,000), which bore the $\gamma 2a$ serotype. Figure 1 shows the result of this experiment. This result is contrasted to the earlier experiment in which another independent clone of the same primary type 45.6 ICR 4.68 (ref. 3) gave rise, on recloning, to clones synthesising heavy chains of molecular weight 40,000. These experiments are summarised in Table 2.

In the second series of experiments, the single primary variant which had been previously characterised and shown to synthesise a $\gamma 2a$ heavy chain of molecular weight 75,000 (45.6.2.4 ICR 9) was recloned and overlaid with an antiserum directed against the Fc region of MPC 11 ($\gamma 2b$) or with an antiserum directed against a $\gamma 2a$ immunoglobulin (LPC-1). Neither antiserum is specific for subclass determinants. Each is reactive with both $\gamma 2a$ and $\gamma 2b$ myeloma proteins. In the initial experiment, one of 660 clones was stained with the anti-MPC 11 Fc serum. This clone was found to be synthesising and secreting heavy chains which continued to bear the $\gamma 2a$ serotype, but were now of decreased (normal) size (molecular weight 55,000). The results are shown in Fig. 1.

In a subsequent experiment with 45.6.2.4 ICR 9, one of 766 clones was stained with the anti- $\gamma 2a$ antiserum. This stained clone continued to synthesise the primary variant heavy chain of molecular weight 75,000. Upon recloning this stained clone, 994 out of 995 clones were unstained, indicating a considerable degree of clonal variability. One of the 995 clones was stained and was now found to be making a $\gamma 2a$ chain of molecular weight 55,000.

In another independent experiment utilising anti- $\gamma 2a$ antiserum, five out of 1,150 clones were stained. Four of the five were identical to the primary variant except that they now secreted the variant heavy chain in sufficient quantity to be detected as stained in the overlay procedure. The remaining clone synthesised a heavy chain of decreased (normal) size (molecular weight 55,000) having the $\gamma 2a$ serotype. This secondary variant was quite stable, since on recloning, all of 3,000 subclones were stained with the anti- $\gamma 2a$ antiserum and 15 subclones examined by radiolabelling and electrophoresis on acrylamide gels were identical in the size and assembly patterns of the heavy and light chains they synthesised.

The primary variants which gave rise to secondary variants have certain features in common. Neither the molecular weight 50,000 heavy chain nor the molecular weight 75,000 heavy chain is assembled well into H_2L_2 and

neither is secreted well from the cell. The assembly patterns of these primary variants are shown in Fig. 1. Note the relative lack of H_2L_2 in panels *a1* and *b1* as compared with the relative abundance of H_2L_2 in panels *a2* and *b2*. The



Fig. 1 Comparison by electrophoresis on sodium dodecyl sulphate-acrylamide gels of assembly patterns of primary and secondary variants. *a1*, 45.6.3.2 ICR 11.19, which synthesises a heavy chain of molecular weight 50,000, and the secondary variant, *a2*, 45.6.3.2 ICR 11.19 S3, which synthesises a $\gamma 2a$ heavy chain of molecular weight 55,000. *b1*, 45.6.2.4 ICR 9, which synthesises a $\gamma 2a$ heavy chain of molecular weight 75,000 and the secondary variant, *b2*, 45.6.2.4 ICR 9.9.2, which synthesises a $\gamma 2a$ heavy chain of molecular weight 55,000. Panels *b3* and *b4* show the respective heavy and light chains after total reduction and alkylation of the disulphide bridges. The assignment of H_2L_2 , H and L is done in accordance with ref. 6 and confirmed by comparative molecular weights from these and other data.

Table 2 Conversion of primary variants to secondary variants

Size and serotype of parent chain	Parent cell line	Mutagen	Primary variant: name and size of heavy chain	Secondary variant: size of heavy chain	Serotype
IgG-2b (55,000)	45.6	ICR-191	(45.6 ICR 4.68) 50,000	40,000	Negative
	45.6.3.2	ICR-191	(45.6.3.2 ICR 11) 50,000	55,000	($\gamma 2a$)
	45.6.2.4	ICR-191	(45.6.2.4 ICR 9) 75,000	55,000	($\gamma 2a$)

diminished ability of some variants (45.6 ICR 4.68, 45.6.3.2 ICR 11.19, and 45.6.2.4 ICR 9) to secrete immunoglobulin heavy chains may confer a selective disadvantage on these cells, thereby allowing us to detect secondary variants in these populations. In contrast, primary variants synthesising heavy chains of molecular weight 40,000 and 55,000 seem quite stable. In the latter case, the heavy chains are assembled well into H_2L_2 (as shown in Fig. 1, panels a2 and b2) and are subsequently secreted from the cell^{3,4}. Consequently, both primary and secondary variants of these stable types are especially valuable since they secrete myeloma protein into the sera of mice bearing tumours induced by subcutaneous or intraperitoneal injection of the variant cells. Thus, structural studies of the variant heavy chains are facilitated.

Many observations suggest that all our variants are the result of mutation. The variants are generally stable and are increased in number after mutagenesis, and the heavy chains they synthesise differ structurally from the parent. In addition, the variants we have seen, whether primary or secondary in origin, seem to fall qualitatively into the four groups shown in Table 1. These groups are defined by size of the heavy chain, assembly characteristics, and serological characteristics even though the members of each group differ somewhat by peptide mapping and, in some cases, by rate of assembly. Perhaps the quantal characteristics of the sizes of variant heavy chains produced will reflect the presence of certain genetic hot spots which are sites of rapid mutation.

On the other hand, the primary variants arise at a remarkably high incidence. For example, treatment of the parental population with $1 \mu\text{g ml}^{-1}$ of ICR-191 yields unstained clones at an incidence of 2%. One-third of these clones are synthesising altered heavy immunoglobulin chains. These incidences are extraordinary when compared to measurements in bacteria and other mammalian cell culture systems. This observation, together with the spontaneous generation of secondary variants from certain primary variants, makes us question whether the original lesion is within the structural gene dictating the parental heavy chain. It is possible, for example, that exposure to certain mutagenic agents will render a cell generally hypermutable because of an interaction with a regulatory element. It is interesting, therefore, that so far variants synthesising heavy chains of molecular weight 50,000 or 75,000, that is, those which have subsequently yielded secondary variants, have been selected only after treatment with ICR-191. The two stable types, those which synthesise short heavy chains of molecular weight 40,000 and those which synthesise γ_2a heavy chains of molecular weight 55,000 have appeared after treatment with either ICR-191 or with Melfalan^{3,4,7}, a phenylalanine mustard used in clinical treatment of human myeloma. Whether particular primary variants may have an essential instability is a question we are actively exploring.

We hope that the combination of cellular studies with the careful structural analysis of variant heavy chains will help us to analyse the genetic and molecular nature of the variations observed in these cells.

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Annihilation of positrons in D and L isomers of various amino acids

GARAY *et al.*¹ have reported on experiments, on positron annihilation in D and L isomers of various amino acids. I show here that one of the two interpretations which they put forward to explain their results is in error, and also discuss other possible experiments which may enable clarification of the rather unusual results they have reported.

In the experiment referred to (paraphrasing the authors' description) 'crystalline samples of D and L amino acids were packed around a 0.8- μCi Na^{22} (positron) source to a thickness of 6-7 mm, and it (source plus amino acid) was placed between two scintillation counters of NE111 plastic scintillator coupled to 56-AVP photomultipliers'. The positrons either annihilate directly, probability >80% and lifetime $\tau \sim 0.2$ ns, or form singlet or triplet positronium (Ps), with lifetimes $\tau_s = 0.1$ ns and $\tau_t \sim 1$ ns (τ_t is reduced from its free space value of 138 ns by electron pickoff). Using standard fast coincidence techniques the authors find the intensity of the long-lived (presumably triplet) Ps components in D and L isomers of a series of 5 amino acids to satisfy the relation $I_L/I_D = 0.67$ (7), 0.72 (6), 0.82 (5), 0.83 (6), 0.94 (6). The numbers in parentheses are experimental errors and the results of 0.67 and 0.82 come from samples of tryptophan produced by different laboratories. The authors conclude from this data that "... D isomers of amino acids favour triplet states in the case of forward polarised β^+ particles. It seems likely from this evidence that β decay was the cause of an initial asymmetry in the racemic mixtures present on the primordial Earth".

The authors interpret these results in terms of a possible correlation between the electron velocity and the electron spin within the sample, $\langle v_a \sigma_B \rangle = K \delta_{aB}$ where K has opposite sign for L and D and $\langle \rangle$ refers to an ensemble average. They also assume that the probability of Ps formation depends on the relative velocity of electron and positron. If then the positrons have a non-zero helicity h ($h \equiv \langle \sigma(e^+) \cdot V(e^+) \rangle$ where $\sigma(e^+)$ is the e^+ spin vector, $V(e^+)$ the e^+ velocity direction, and $\langle \rangle$ refers to an ensemble average over the e^+ beam) the probability of triplet against singlet Ps formation will vary in switching from L to D. Thus one would expect that the intensity of the short and long components in the time spectrum should depend on the isomer under consideration. Note that in this interpretation it is the helicity, not the polarisation $P \equiv \langle \sigma(e^+) \rangle$ of the beam, on which I_L/I_D depends. As an instructive example to illustrate this point consider the limiting (and completely artificial) cases in which Ps formation occurs only for $V(e^-)$ parallel to $V(e^+)$ and for $K = +1$ for L and -1 for D. If we now note that the spinor direct products $\uparrow\uparrow$ or $\downarrow\downarrow$ ($\uparrow, \downarrow; \uparrow, \downarrow$ refer to e^-, e^+ spins) give half triplet and half singlet decays one may easily show that the situation $P=0$ for $h = +1-1$ yields $I_L/I_D = 2$ or 0.5 while $h = 0$ yields $I_L/I_D = 1$ for any P.

We now remark that whatever the correlation between Ps formation and relative velocity and whatever the actual (presumably small) value of K is, $h \approx 0$ at e^+ kinetic energies (~ 5 eV-15 eV) characteristic of Ps formation. This can be demonstrated by noting that experimental² and theoretical^{3,4} studies have shown that if a beam of electrons (or presumably positrons) impinges on a surface with energy E_0 (10^4 eV $< E_0 < 10^6$ eV) the beam will have a completely diffuse nature (velocities

completely random) by the time it has penetrated a distance where the mean energy of its constituent particles is still $>3,000$ eV. This may be most readily arrived at by applying formulas (4) and (5) of Archard⁴ for the case $Z \sim 3-10$ (the result is insensitive to Z). Since at energies $\leq m_0c^2$ ($=0.5$ MeV) σ does not follow V (see ref. 5) it follows that $h \approx 0$. Note that the energy range 10^4-10^6 eV brackets the energy of almost all e^+ emitted by ^{22}Na . In addition, though it is not relevant to the problem, we point out that the geometry of the experiment gives $P \approx 0$ as well.

We conclude that the effect seen by Garay *et al.* is in all likelihood not a result of positron polarisation or helicity. If real it may be a result, as Garay *et al.* recognise, of the inhibition of Ps formation and/or greater triplet quenching in L as opposed to D, possibly because of an undetected chemical impurity. It may also be caused by some slight difference between the solid state structure of the L and D crystals. If so, a new simple, non-destructive and highly sensitive method of detecting such impurities is now available. Experiments to determine the dependence (or lack thereof) of I_L/I_D and the overall Ps formation rate on P and possibly on h are now being started. To try to investigate the dependence of I_L/I_D on h we may use the low energy positron beam available in our laboratory, if the polarisation of the beam is sufficiently high.

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Conformations of double-helical nucleic acids

A KNOWLEDGE of possible conformational states of double-helical nucleic acids is fundamental to an understanding of how they work^{1,2}. Models for a series of conformations have been made on the basis of X-ray data³⁻⁵. Investigations of nucleic acids in solution point to the existence of a continuum of forms but do not make possible the quantitative determination of the conformational parameters. Here, theoretical investigation of the intramolecular interaction energy as a function of conformational parameters and a search for minimal energy regions in the space of these parameters become essential.

Two families of nucleic acid conformations are known, the A and B families, which differ by the mutual position of the base pairs, the conformational angles in the ribose-phosphate backbone and the conformation of the ribose ring. This last is a major factor determining whether a double-helical structure belongs to the A or B family of conformations. The C_2' -endo conformation in the A family and the C_2' -endo or C_3' -exo conformation in the B family are assumed⁴. Data on the conformation of the ribose ring cannot be obtained from the diffraction patterns of nucleic acid fibres. The ribose conformation is taken from X-ray data for the nucleic acid components. The ribose ring in double-stranded helical nucleic acids is, however, in different surroundings from that in monomer crystals. It may be rearranged in the course of transitions between both the families and the different forms of a family. In this connection, the intramolecular interaction energy of double-helical nucleic acids was considered in our work (unlike the recent

works^{6,7}) with an account of conformational possibilities of a ribose ring.

We calculated the intramolecular interaction energy for the regular double-helical polynucleotide containing rigid Watson-Crick pairs as a function of eight conformational variables. Four of them determine the mutual position of the base pairs (D is the distance from the pair centre to the helical axis, d the pair-pair distance along the helix axis, θ the tilt angle of the base pairs to the plane perpendicular to the helix axis, τ the rotation angle of one pair with respect to another around the helix axis), four others ($OC_1'C_2'$ and $C_1'C_2'C_3'$ bond angles and dihedral angles τ_1 and τ_2) determine the conformation of a ribose ring. The bond angles of a ribose ring were considered as variables in contrast with other bond angles. The bond lengths were fixed. The eight variables with the above-mentioned

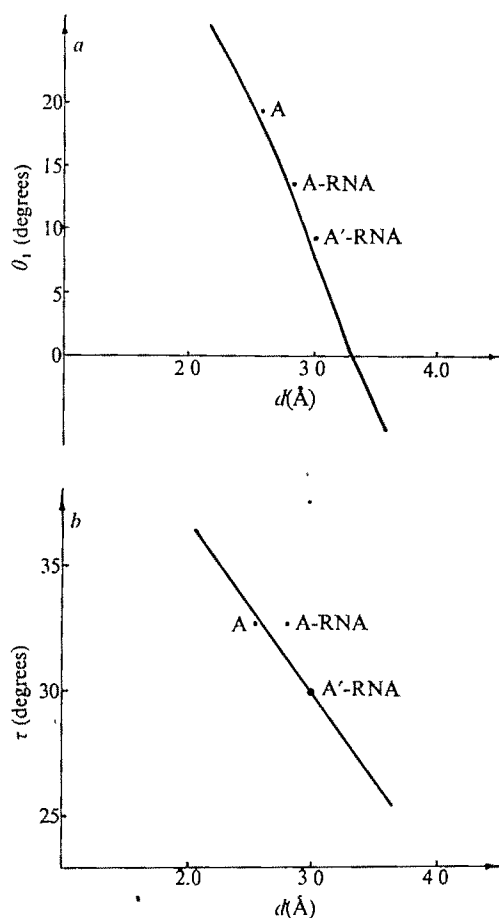


Fig. 2 Projections of the B-family bottom position for the poly(dA).poly(dU): a, on the (D , θ); and b, on the (D , τ) planes. The known forms are marked.

constraints completely determine the conformation of a regular polynucleotide.

Dihedral angles of sugar-phosphate backbone were determined by means of a procedure which resembles one of Go and Scheraga's⁸. The van der Waals' interactions were calculated by the atom-atom potential function method⁹. The Lennard-Jones potentials were used; the validity of parameters was verified by calculations on nitrogen base crystals. The heats of sublimation and lattice constants of a number of aromatic compound crystals were reproduced (V.I.P., unpublished). The tension energy of the bond angles in the ribose ring was assumed to be proportional to the square of the deviation of these angles from the tetrahedral ones. Previously¹⁰ we studied the interaction energy between the base pairs as a function of parameters determining their mutual position in a double helix. We detected a limited region in the space of these parameters

energetically allowable with these parameters. Thus the region of the eight-dimensional conformational space which should be investigated might be drastically diminished. The many-variable function minimisation procedure was used for searching for the energy minima.

The calculations showed the existence of two valley-like regions of minimal values on the energetic surface. One of them corresponds to the A family of nucleic acids, the other to the B family. The energy variation along the bottom of each valley is rather small; the energies are the same for both valleys up to 1 kcalorie mol⁻¹. The points which correspond to the models constructed by means of X-ray data are placed in the conformational space near the lines which describe the position of the bottom of a valley. There is a systematic deviation towards the lower D values for the points which correspond to the models of the A forms. But if $D = 4.7$ Å is fixed (like the models of the A forms) the line in the conformational space which corresponds to the bottom of this valley passes through the experimentally determined forms of the A family. This displacement is connected with the intramolecular interaction energy change by 1–2 kcalorie mol⁻¹. It is not excluded that the double-helical macromolecule contracted in diameter due to intermolecular interactions with neighbouring molecules in fibres. The A family is characterised by a nearly linear relationship between the parameters d and θ as well as d and τ (Fig. 1). The forms both with great and with small distances between the pairs along the helix axis are sterically allowed. The position of the valley bottom corresponding to the B family is characterised by the constancy parameter d (approximately 3.4 Å). For the B family, τ increases almost linearly whereas θ strongly diminished with the decrease of D in accordance with the

Fig. 1 Projections of the A-family bottom position for the poly(dA).poly(dU): a, on the (d , θ); and b, on the (d , τ) planes ($D = 4.7$ Å). The known forms are marked.

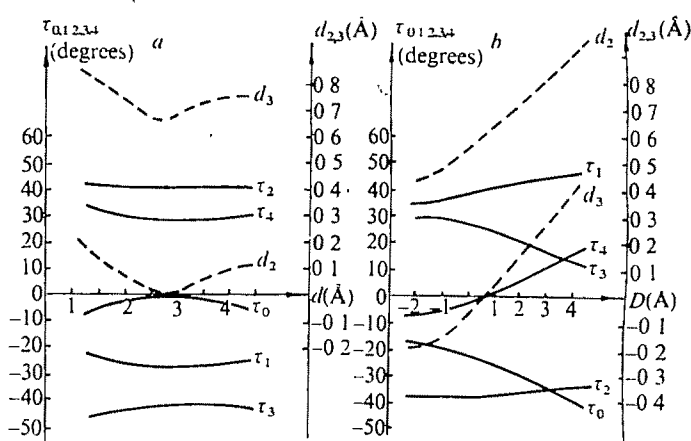
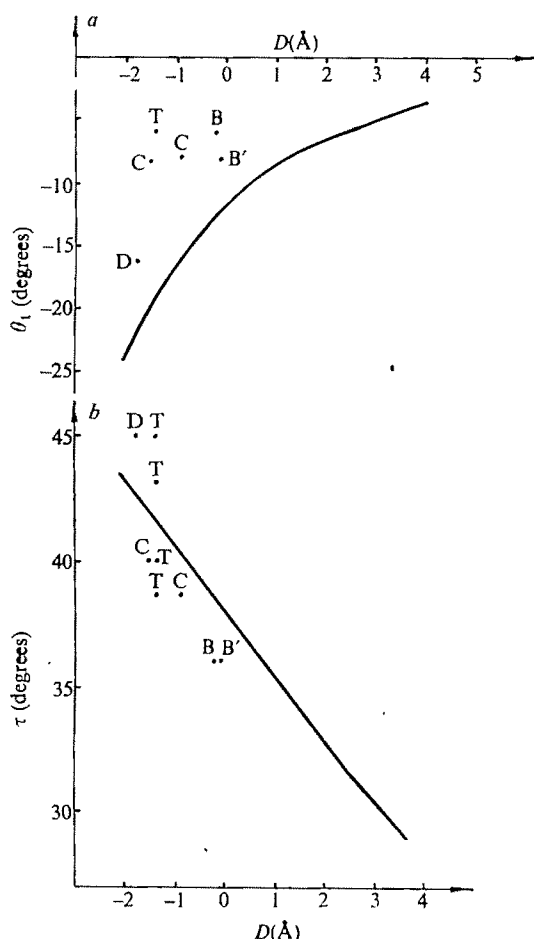


Fig. 3 Dependence between the changes in the conformational parameters of deoxyribose ring and the position of the structure on the valley bottom for A (a) and B (b) families. $\tau_0, \tau_1, \tau_2, \tau_3, \tau_4$: rotation angles around $O'-C_1', C_1'-C_2', C_2'-C_3', C_3'-C_4', C_4'-O'$ respectively; d_2 and d_3 : the derivations of the C_2' and C_3' atoms from the plane of other atoms of a sugar ring. The positive values corresponds to deviation towards C_5' atom.

results of X-ray studies (Fig. 2) A systematic deviation towards the lower θ values can be seen for the points which correspond to the models of B family forms but there is a tendency to raise absolute value of θ in recent models

A correlation is observed between the disposition of the base pairs which corresponds to the optimal energy values and the sugar ring conformation (Fig. 3). The last one resembles the standard C_4' -endo or C_3' -endo conformation for those parts of valleys where models based on X-ray studies exist. The ring deformation in the B family is more pronounced than that in the A family. At the positive D values both C_2' and C_3' atoms deviate from the $C_1'O'C_4'$ plane towards C_5' (Fig. 3b).

An energetically unfavourable region exists in the conformational space between the A and B family valleys. The minimal steric barrier between the two families is about 3 kcalorie mol⁻¹.

The relationships between the conformational parameters of a double-helical polynucleotide which we have obtained may be used to detect the degrees of freedom of the system for the analysis of its behaviour under the effect of weak perturbations (as against intramolecular interactions). Different double-helical nucleic acid conformations may exist in different conditions in the interaction with some biologically important compounds.

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matters arising

Particle creation and Dirac's Large Number Hypothesis

In a recent paper¹ Steigman claims to show that the creation of matter as postulated by Dirac² is unnecessary. In particular he claims that a theory with a gravitational constant G varying as t^{-1} automatically implies that the number of nucleons within the horizon should increase as t^2 . If so, Dirac's hypothesis that $N \sim t^2$ must be due to spontaneous matter creation becomes unnecessary. We here show Steigman's claim to be incorrect.

First, note that within Dirac cosmology the present radius of the Universe varies as $R \sim t$. Assuming such a time dependence holds in the very early Universe, as Steigman does, then there is no horizon since the integral

$$d_H(t) \equiv cR(t) \int_0^t \frac{d\tau}{R(\tau)} \quad (1)$$

defining the horizon distance diverges logarithmically. Therefore, any increase of mass, M , with time, t , no matter how, must be ascribed to spontaneous creation since no matter is entering the space, V , across the expanding horizon.

We now show how matter creation must be an independent hypothesis for Steigman's equation (1) to be correct. As shown in ref. 3, the correct Einstein equation in atomic units is given by

$$\left[\frac{\dot{R}}{R} + \frac{\dot{\beta}}{\beta} \right]^2 + \frac{k}{R^2} = \frac{8\pi}{3} \bar{G} \bar{\rho} \beta^2 \quad (2)$$

Here β is the gauge function which varies asymptotically as t^{-1} , and \bar{G} and $\bar{\rho}$ are given in Einstein units. In Einstein units \bar{G} is constant, and $\bar{\rho}$ is given by

$$\bar{\rho} = \bar{M} / \bar{R}^3 \quad (3)$$

where, following Dirac², \bar{M} and \bar{R} are constant so $\bar{\rho}$ is constant. Since $\bar{R} = \beta R$, where \bar{R} and R are the radii of the Universe in Einstein and atomic units, respectively, equation (2) becomes

$$G\bar{\rho}\beta t^2 = \text{constant} \quad (4)$$

where we have used $G = \bar{G}\beta \sim t^{-1}$ and

$R(t) \sim t$. Equation (4) coincides with Steigman's equation (1) only if we define

$$M/R^3 \equiv \rho \equiv \rho\beta \equiv \bar{M}\beta/\bar{R}^3 \quad (5)$$

Since $\bar{R} = \beta R$ it follows that the mass entering the definition of ρ , that is, M , must be related to the constant mass \bar{M} by the relationship

$$M = \bar{M}/\beta^2 = \bar{M}t^2 \quad (6)$$

which explicitly shows matter creation.

The error in Steigman's reasoning lies in the postulate of his equation (1) which does not have the backing and theoretical framework that justifies and correctly defines each quantity in the equation. One cannot simply postulate the existence of a relationship of the type

$$G\rho t^2 = \text{constant} \quad (7)$$

and then take $G \sim t^{-1}$ assuming, as Steigman does, that ρ does not contain matter creation. If such a theory exists, as it might well do, it cannot be presented under the name Dirac cosmology. In fact, the full Dirac theory yields a relationship like equation (7) only if ρ is defined by equation (5), that is, if it already contains matter creation.

The second part of Steigman's criticism is invalid for a different reason. Here he points out that Dirac's large Number Hypothesis (LNH) implies such things as the fact that there can be no primordial nucleosynthesis, and that for $T \sim 10^{10}$ K the number of baryons in the Universe is of order unity.

In fact Dirac² has repeatedly stressed that the LNH is an asymptotic theory valid only for relatively large times over most of the age of the Universe, but not necessarily at very early epochs. Thus the second part of Steigman's paper strongly supports Dirac's statements and in no way invalidates the LNH.

In summary, Steigman's claim that Dirac's LNH does not require particle creation is wrong because he has assumed that which he was seeking to prove, that is that ρ does not contain matter creation. Steigman's claim that Dirac's LNH leads to nonsensical results in the very early Universe is superficially correct, but this only supports Dirac's contention that the LNH may not be valid in the very early Universe.

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¹ Steigman, G., *Nature*, 261, 479 (1976).

² Dirac, P. A. M., *Proc. R. Soc.*, A338, 439 (1974).

³ Adams, P. J., Canuto, V., Hsieh, S. H. and E. Tsiang, *Phys. Rev. D* (in the press).

STEIGMAN REPLIES—In Dirac's original cosmology the scale factor varies as $R \sim t^{1/3}$ (there is a possible confusion by Canuto *et al.* between 'scale factor' and 'radius of the Universe'). In this model there is a horizon and the results and conclusions of my paper apply. There are, of course, a large number of possible modifications of Dirac's original model. In the variation chosen by Canuto *et al.*¹ ($R \sim t$) there is no horizon. In this model then, there are always an infinite number of particles 'in the Universe' and, clearly, Dirac's Large Number Hypothesis (LNH) cannot apply.

Canuto *et al.* argue that several of my criticisms of Dirac's theory are invalid because the LNH is an 'asymptotic' theory. It is certainly not clear why this should be so; why do the equations in Canuto *et al.* not apply for all times? A cosmological theory which only predicts the present epoch is of questionable value.

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¹ Canuto, V., Adams, P. J., Hsieh, S. H., and Tsiang, E., *Nature*, 263, 485 (1976).

The pre-palaeozoic basement in south-eastern Scotland and the southern uplands fault

BASAMENT xenoliths in Carboniferous volcanics in the vicinity of Partan Crag along the south side of the Firth of Forth have been used to infer the character of the immediately underlying basement by Upton *et al.*¹. These

xenoliths are probably representative of a subjacent basement but they cannot be unequivocally regarded as reflecting the basement character beneath the geological Midland Valley of Scotland, in a larger structural sense, as has been argued. Although the south-east boundary of the Midland Valley, as a geological feature, has been commonly taken along the Lammamuir Fault², it is likely that this fault may only be a shallow and relatively insignificant feature at depth. The magnetic map³ shows that the major geophysical break or linear, which, to the west, follows the course of the Southern Uplands Fault, passes near to or north of Partan Crag. The prominent magnetic linear lies everywhere between the Southern Uplands Fault and the faults associated with the structural hinge that passes from near Girvan to Edinburgh⁴. This suggests that either or both of these structures on the surface reflect the true position at depth of a significant crustal structure, which should be termed the Southern Uplands Line. It is suggested that the use of the Southern Uplands Fault alone as a single line taken as marking the surface expression of a major deep seated structure is unrealistic.

Along the south-east part of the Midland Valley, the magnetic linear³, which along the south-west margin is nearly coincident with the Southern Uplands Fault, diverges to the north from the geological southern margin of the Midland Valley in the vicinity of Dalkieth and passes near the southern margin of the Firth of Forth. There is a north-south distance of up to 18 km between the Southern Uplands Fault to the south, and the southern margin of the structurally more significant magnetic linear. The broad, open pattern of extensive, regular magnetic signature with shallow gradients, which can be traced through the Southern Uplands³, clearly extends beneath and North of the Lammamuir (Southern Uplands?) Fault and strongly suggests that rocks which are magnetically characteristic of those below the Southern Uplands pass northwards to at least the magnetic linear zone. This recognition of the probable northwards extent of the Southern Uplands basement would place the Southern Uplands basement very near to Partan Crag. It could then be argued that the basement rock types noted by Upton *et al.*¹, probably are characteristic of the basement beneath the Southern Uplands. This would be consistent with the basement character suggested from the western end of the Longford-Down Massif in Ireland⁵, which is a geophysical and probable geological extension of the Southern Uplands^{6,7}.

As it is uncertain whether the in-

ferred pre-Dalradian basement of Upton *et al.*¹ underlies the Midland Valley or the Southern Uplands tectonic zones, it would probably be unwise to be too specific about structural reconstructions involving the precise location of the landmass lying to the

Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

south of the Dalradian sedimentary basin. The relationship of the xenoliths to the basement area they have been derived from must first be established with greater precision. If there is a significant difference between the basements beneath the Midland Valley and the Southern Uplands in SE Scotland, and this is not altogether certain, then the xenoliths could probably be better regarded as representing the basement to the south of the Southern Uplands line, rather than that to the north, beneath the Midland Valley.

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Running Dinosaurs

ALEXANDER'S¹ use of stride length and footprint size to determine from trackways the speed of terrestrial vertebrates will greatly increase our understanding of dinosaurian behaviour. None of the trackways he cited were of large running dinosaurs, although he did not suggest that large dinosaurs never ran. We herein cite one such example, and another of a very fleet dinosaur that was not running.

In a trackway of a giant bipedal ornithischian from the Cretaceous (Campanian) of Colorado² the length of the footprint (l) is 0.86 m and the stride (λ) is 9.25 m. Similar footprints have been referred to the Hadrosauridae, the only large bipedal ornithischians then present in North America (ref. 3: footprint in collections of Drumheller and District Museum, Alberta). Combining Alexander's method to find hip height (h ; $h=4l=3.44$ m) with Colbert's⁴ method for estimating the weight of an *Anatosaurus* specimen (where $h=2.25$ m), it is apparent that the Colorado animal weighed about 11 tonnes or as much as two bull African elephants combined. This exceeds by a factor of nearly three the weights obtained for hadrosaurids from the northern interior of North America⁵, but is less than what we estimate (13.8 t) for a giant lambeosaurine hadrosaurid from Mexico⁶. We obtained our estimate using Colbert's⁴ method and considering a 1/12 scale model of *Hypacrosaurus altispinus* (NMC 8501) with a volume of 1,372 ml as a 1/17.5 scale of the Mexican lambeosaur (based on humeral lengths in the two specimens). According to Alexander's formula, the Colorado animal was running at a velocity of 7.54 m s^{-1} (27.1 km h^{-1}).

Anatomically, it has been suggested that some ornithomimids weighing about 140 kg could attain speeds of 80 km h^{-1} (ref. 6). (A 1/6.5 scale model of the ornithomimid *Dromiceiomimus breviterius* (NMC 12228) has a volume of 564 ml, yielding a weight of 154.9 kg, using Colbert's⁴ method.) In an ornithomimid trackway from the Campanian of Alberta⁷ λ is 1.88 m and we estimate h to be 1.22 m, indicating a speed of 1.77 m s^{-1} (6.4 km h^{-1}). According to Alexander¹, this animal would have begun to run when λ/h reached 2, or at a speed of 2.75 m s^{-1} (9.9 km h^{-1}). At a speed of 80 km h^{-1} , prints from the same foot (λ) would be 8.6 m apart.

In our opinion, trackway data so far obtained are not yet adequate for generalisation on dinosaurian metabolism.

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reviews

Membrane-bound glycoproteins

Gilbert Ashwell

Membrane Glycoproteins. A Review of Structure and Function. By R. Colin Hughes. Pp. 367. (Butterworth: London and Boston, Massachusetts, March 1976.) £15.00.

FOR years, the presence of carbohydrates covalently linked to protein molecules was regarded with disbelief, dismay, or even indifference, by classical protein chemists intent on structural determination. Eventually, as a result of the brilliant pioneering studies of men such as Dische, Neuberger, and Winzler, delineation of the chemical bond between carbohydrates and proteins was accompanied by a growing awareness of the role of surface glycoproteins as 'informational macromolecules' mediating a variety of biological phenomena on a cellular level. As a consequence, the past decade has seen a logarithmic growth in the number of publications documenting the ubiquitous presence and complex interactions of membrane-bound glycoproteins.

It is to this problem that the author has addressed himself in a valiant and impressive effort to organise, systematise, and summarise the diffuse and multidisciplinary reports which comprise the current literature on glycoproteins. It is, perhaps, the latter consideration which epitomises the difficulty in maintaining an integrated perspective on the relevance of findings in widely diverse scientific disciplines. Few investigators, actively pursuing their immediate research interests, are either willing or able to devote the time and effort necessary to master the vast amount of potentially relevant information available in journals and disciplines with which they have an only peripheral acquaintance.

Fortunately, that task has been brilliantly accomplished in this monograph. Beginning with a detailed examination of the methodology used in the detection and distribution of membrane glycoproteins, a number of well referenced tables are provided to summarise the important properties and chemical composition of plasma membranes isolated from various species, the surface characteristics of the commonly used tissue culture lines, and the availability of specific glycosidases useful in the analysis of terminal carbohydrate residues. Although

the major thrust of current studies on cell surface glycoproteins has been directed mainly at the outer surface of the cell, the membranes of the sub-cellular organelles are similarly rich in glycoproteins, and their participation in intracellular phenomena is largely speculative. This problem is considered in some detail in a provocative chapter which summarises the available information and seeks to interpret current views on the genesis of primary and secondary lysosomes.

Following this, the role of oligosaccharide determinants involved in human blood group activity and the chemical nature of the ABO and MN antigens is reviewed. The judicious use of illustrative diagrams and chemical formulae facilitate comprehension of the necessarily cumbersome nomenclature used for carbohydrate chains. This section also provides a good overview of the chemistry of the histocompatibility antigens which is quite adequate for the non-specialist reader; extensive referencing is included for those seeking more detailed information. A minor point that requires comment is the statement (p126) that histocompatibility antigens are precipitated by anti-H antisera. To the best of this reviewer's knowledge this is not quite accurate in that a second precipitating agent is always required.

The middle portion of the book covers the extensive literature on lectins and their fascinating reactivity

toward the membrane glycoproteins of the lymphocyte, with emphasis on the biological implications of cellular transformation. Finally, the current status of the biosynthetic and degradative mechanisms involved in the overall metabolism of glycoproteins is presented in elaborate detail. Especially noteworthy is the section on lipid intermediates which is written with commendable lucidity and insight. The discussion on the role of specific carbohydrates in the 'recognition' phenomena is thoughtful and well-documented. The speculation (p297), however, that the hepatic binding protein participates as a glycosyltransferase in the adhesive properties of membranes, as described by Roseman, is unlikely since the purified binding protein was shown to be devoid of both sialyl- and galactosyl-transferase activity.

In summary, this monograph provides an impressive synthesis of the glycoprotein literature up to and including 1974. The author has earned the gratitude of innumerable investigators in diverse disciplines, who are concerned with the biochemical basis of membrane function and its role in cell biology.

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Membrane recipes

Biochemical Analysis of Membranes. Edited by A. H. Maddy. Pp. ix+513. (Chapman and Hall: London; Halsted: New York, July 1976.) £16 50.

THIS is a most timely book. In recent years a considerable expertise has grown up in membrane biology. But all the expertise in the world is wasted unless studies are carried out using pure samples of known origin. For studies of molecular events in membranes, the problem boils down to the isolation and characterisation of single membrane fractions with minimal loss of intrinsic components or gain of extrinsic components. Although progress in this direction has been fairly rapid, the development of suitable procedures

has remained more of an art than a science: hence the need for a book of this kind.

What Dr Maddy has done is to persuade a number of authors to present background discussion and experimental recipes for the preparation and analysis of the most common membranes and membrane constituents. The general format of each chapter is excellent. The first part consists of a comparative literature survey and the second of fully detailed experimental procedures, aimed largely at the level of the novice. The only real test of a collection of recipes is, of course, to try them in the kitchen, and this I have not yet had a chance to do. The couple of procedures published here

with which I am familiar, however, include all the useful tricks, and the others have an authoritative look about them.

The first section of the book consists of procedures for the isolation of membranes, and includes chapters on Mycoplasma membranes (Razin and Rottem), surface membranes (Neville), mitochondrial membranes (Sottocasa), endoplasmic reticulum (De Pierre and Dallner) and nuclear membranes (Harris and Agutter). The only pity here is that there is nothing on membranes of bacteria such as *Escherichia coli* or on micro-organisms such as *Tetrahymena*, both of which are proving of importance in studying correlations between membrane structure and function.

The second section of the book is concerned more with the analysis of membranes, and includes chapters on the solubilisation of membranes (Maddy

and Dunn) and on the analysis of membrane proteins (Dunn and Maddy), lipids (Veerkamp and Broekhuysse) and carbohydrates (Cook). These are followed by some more peripheral, but no less interesting chapters on the applications of phospholipases (Zwaal and Roelofsen), on immunochemical gel precipitation techniques (Bjerrum and Bog-Hansen) and on protein labelling (Hubbard and Cohn).

It is perhaps a sign of how far the study of membranes has progressed that a book of this kind can be written; and there can be few working with membranes who would not gain by browsing through it. Finally, there is a coherence about the book which it is rare to find in an edited volume, and for which the editor deserves high praise.

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Transition metal compounds

Methodicum Chemicum Volume 8 Preparation of Transition Metal Derivatives. Edited by Kurt Neidenzu and Hans Zimmer. Pp. x+579. (Academic: New York, San Francisco and London, 1976.) \$110; £67.10.

THIS text details published and proven methods for the preparation of transition metal compounds. In general, one chapter is devoted to each of the thirty d-transition elements; the lanthanide and actinide groups of elements each merit one chapter, a summary of ferrocene chemistry is included; and aspects of carbonyl, metallocene, and heteropolyanion chemistries are reviewed separately. The material for each chapter is classified in order of increasing oxidation state, a reasonably good index is provided and no real difficulty should be encountered in using the volume as a reference source. Each chapter is referenced in a reasonably comprehensive manner and nearly 7,000 original publications and review articles are cited in all. Some 29 authors have made one or more contributions, and, in several instances, these chemists are internationally recognised as authorities on their chosen topic.

Despite the considerable collective talent and effort involved in the preparation of this work, however, several reservations must be expressed when considering a recommendation to purchase this expensive volume. The most serious of these reservations results from the inherent nature of this work, which attempts to describe contemporary synthetic transition metal chemistry

in less than 600 pages. Since the space devoted to a particular element is no more than fifteen pages, the book reads like a laundry list. The description of the preparation of any particular compound, or group of compounds, is covered in one sentence of the 'add A to B to produce C' variety, with no details of the yield, purification procedure(s), necessary precautions, or properties of the compound(s) being given. As none of the material is original and the authors have been critical only in their selection of the material to be included, much of the information presented is readily accessible to the initiated inorganic chemist in standard and specialist texts and review articles. These criticisms are all the more serious since, due to certain unspecified difficulties, a considerable delay has occurred between the submission of the manuscripts and their publication, so that the coverage of the literature ends at 1970.

This volume is an extremely useful, if somewhat outdated, reference source for synthetic routes developed for the preparation of a wide range of transition metal compounds. In view of the reservations outlined above, the inorganic research chemist could spend the amount of money involved more wisely by purchasing a collection of other texts. The non-specialist inorganic chemist, and other scientists interested in the preparation of inorganic compounds, should, however, find this compilation of considerable value.

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Electrophilic halogenation

Electrophilic Halogenation. By P. B. D. de la Mare. Pp. xi+231. (Cambridge University: Cambridge, 1976.) Hardcover £10.50; softcover £4.50.

ALTHOUGH some discussion of the importance and mechanism of electrophilic halogenation is to be found in most undergraduate texts, there is a need for an up-to-date, detailed and unified discussion of such reactions.

In this book electrophilic halogenation is covered from a mechanistic viewpoint. It is a complex area where the problems of reactivity, regioselectivity, stereoselectivity, product multiplicity and the nature of the halogenating species all have to be taken into account. The author, who has been an active research worker in this area for some thirty years, adopts an approach which considers the formation and fate of the carbonationic species produced by attack of electrophiles in general on unsaturated compounds, both aromatic and aliphatic. The basic patterns which emerge are then elaborated for each of the halogens and their derivatives in turn. Topics such as the acid and base catalysed α -halogenation of carbonyl compounds are covered in a chapter on miscellaneous electrophilic halogenation, and there is also a chapter on electrophilic halogenation of aromatic heterocycles. Introductory chapters provide the necessary background in physical organic chemistry and describe the halogens and their derivatives.

In keeping with the Cambridge University Texts philosophy, the level of the book is intended to suit final year undergraduate and early postgraduate students. The book contains many references to the original literature and is clearly written. It will nevertheless be demanding at the level intended, concentrating as it does on physical organic rather than descriptive aspects of the subject. The undergraduate may also feel that the area of chemistry covered for the price of the book is somewhat limited; and indeed the book may have had a wider appeal if the scope had been broadened to cover either electrophilic addition and substitution in general, or halogenation in general. The latter suggestion has much to commend it since free radical halogenation is also very important and frequently competes with the electrophilic process.

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Protein polymerisation

Thermodynamics of the Polymerization of Protein. (Molecular Biology: An International Series of Monographs and Textbooks). By Fumio Oosawa and Sho Asakura. Pp. viii+204. (Academic: London and New York, March 1976) £6 80; \$16.75.

NATURE constructs the elementary blocks composing the constituents of living matter from small inanimate molecules which abound in our environment. The synthesis of the building blocks as well as of the highly specific functional macromolecules which they eventually form, is a complex process. It proceeds with the aid of elaborate machinery, specific templates and an ample supply of energy. Classical biochemistry and molecular biology study this elaborate process of creation and of disposal of biological macromolecules. It may have come as a surprise that spontaneous assembly of biological structures, unassisted by accessory means, could proceed as well. Indeed there exists an enormous variety of structures which form spontaneously from simpler protein constituents. For this process, which is often reversible and satisfies the laws of thermodynamics, Caspar and Klug have coined the term self-assembly.

The book by Oosawa and Asakura illustrates the impressive contribution of the Nagoya school to the problem of protein polymerisation. In addition to thermodynamics, kinetics, structural requirements, allosteric control, polymorphism and internal motility and flexibility of assembled structures are treated as well. Unfortunately, the bulk of the discussion is restricted to studies of actin and flagellin polymerisation from the authors' and other, not too distant, laboratories. About 76 entries in the authors' index are only perfunctorily listed in the list of references at the end of the volume. Although this represents a useful sectioned compilation, it does not provide the critical discussion (on varied approaches to an interesting problem) one expects from an authoritative monograph. For instance, on page 60 it is stated that in the trimer-disk transformation of TMV protein the enthalpy increases by about 77 kcalorie mol⁻¹, and another experiment gives 14–38 kcalorie mol⁻¹ for the same process. The possible causes of this discrepancy, deriving from the analysis of the dependence of equilibrium constants on temperature on the one hand and calorimetric measurements on the other hand, are not discussed.

In chapter 10, the reader not familiar with the subject may be misled with respect to the chronological development of the use of quasi-elastic light scattering in the study of the dynamics of macromolecular structures. I have doubts whether the reader to whom this monograph is addressed will easily follow the calculations in chapters 6 and 10, and appreciate their significance in terms of the experiments performed. The symbols in Table 3 on page 87 are intelligible to crystallographers and the statement on page 97—"Thermodynamic analysis of the polymerisation equilibrium of sickle cell hemoglobin is not enough to be compared with the above result"—is cryptic.

There are some printing errors, but not excessively many. The illustrations are clear and attractive. The cost is reasonable and should not prevent interested libraries as well as individuals from acquiring this compact, if somewhat one-sided, introduction to a topical field.

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Below 1 K

Refrigeration and Thermometry below One Kelvin. By D. S. Betts. Pp. x+283. (Sussex University: Sussex, 1976.) £8 00.

ONE of the most surprising things about research at very low temperatures, to the uninitiated, is its continuing interest and richness. With each further improvement in technology, enabling physicists to push even closer to their unattainable goal of the absolute zero of temperature, there have emerged new and fascinating phenomena to be observed, quantified, and pondered about. The most significant area of technical development over the past decade has undoubtedly been that of 'He-He dilution refrigeration, which has now reached the point where commercially available machines are capable of maintaining a sample indefinitely at temperatures near 10 mK, thus providing a convenient starting temperature from which the 1 mK region becomes immediately accessible through the addition of, for example, an adiabatic demagnetisation stage. Near 1 mK, of course, are the extraordinarily complex 'new phases' of liquid ³He which, with their superfluidity, magnetism and anisotropy, will continue as a challenge to the physicist

for many years to come. At still lower temperatures, in the as yet virtually unexplored region around 0.1 mK, there is the beckoning prospect of nuclear ferromagnetism.

Dr Betts' book, explaining how to do the experiments, is intended as an introduction for MSc and PhD students, and other beginners whose research involves making measurements below 1 K. The first half of the book describes how to achieve these low temperatures, and consists of six chapters covering ³He and ⁴He evaporators, ³He-⁴He dilution refrigerators, Pomeranchuk cooling, and the adiabatic demagnetisation of paramagnetic salts and nuclei. Less popular techniques such as vortex refrigeration have (not unreasonably) been omitted. Having produced a low temperature the next problem is, of course, that of measuring it. Accordingly, the second half of the book is devoted to a detailed survey of the numerous methods which are available, involving measurements of vapour pressure, paramagnetic susceptibility, nuclear magnetic resonance, electrical resistance, capacitance, thermoelectric e.m.f., electrical noise, the Mossbauer effect, and anisotropic radioactive decay.

The various techniques are outlined with impressive clarity, including a useful amount of mathematical detail, and there are copious references both to review articles and also to the original literature. To keep one's feet firmly on the ground, the book is permeated with valuable numerical information both in graphical and in tabular form, the extensive compilation of data on commonly used paramagnetic salts being a particularly striking example. The index is skimpy, but the careful and logical arrangement of material means that elusive topics can usually be traced fairly rapidly by consulting the table of contents. There is inevitably a substantial overlap in coverage with O. V. Lounasmaa's *Experimental Principles and Methods Below 1 K* (Academic, London 1974). Quite apart from its somewhat different style and emphasis, however, Dr Betts' book has the advantage of being two years more up to date, a factor not to be ignored in this rapidly developing field.

The book succeeds admirably in its stated purposes. It will also be a valuable source of reference for everyone working below 1 K, and most especially for those aspiring to join, in Dr Betts' phrase, "the new army of nuclear coolers... marching into the submillikelvin region."

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Genes or environment

Heredity, Environment, and Personality: A Study of 850 Sets of Twins By John C. Loehlin and Robert C. Nichols. Pp. xii+202. (University of Texas. Austin, Texas and London, 1976.)

MODEST numbers of twins, carefully sampled and personally investigated in clinical detail, sometimes over a period of years, have an acknowledged place as a valuable if limited research method, particularly in the field of psychiatric abnormality. Attention has recently been drawn, however, to the need for large samples for the adequate study of genetic and environmental influences on ability, personality and interests in the normal population. The present study reports data on some 2,000 variables collected from 850 pairs of twins, not one of whom was personally seen by the authors. The information was obtained from examination results and from questionnaires sent by post to the twins and their parents.

The subjects were those twins who participated in the National Merit Scholarship Qualifying Test taken each year by about 600,000 American high school students at the age of 16 or 17 and who completed the postal questionnaires. The authors clearly discuss the effect of possible biases caused by diagnosis of zygosity by questionnaire, selection for academic ability and willingness to cooperate, and the usual excess of identicals and females found in such samples.

They present data and discuss differences between twins and non-twins, and examine in detail early environmental experiences such as dressing alike, parents treating the twins alike, and interpersonal relationships. None of these had much, if any, influence on the resemblance found. The authors conclude that the environment "operates in remarkably mysterious ways, given traditional views on personality and motivational development".

Not surprisingly, identical twins were no more alike than fraternal twins in matters such as race—or their opinions about racial integration. In most measures, however—whether single items, clusters, or the scales of a test such as the California Psychological Inventory—identicals were usually correlated about 0.20 higher than fraternal twins. On ability measures, intrapair correlations clustered around 0.75 and 0.55 in the two kinds of twins, personality measures, 0.50 and 0.30, and measures of self-concept and interests, 0.35 and 0.15.

The authors' main conclusion, that the almost ubiquitous genetic influence is roughly equal in degree for all traits, is admittedly controversial. Furthermore, some of the statistical analyses require critical interpretation, and little attention is given to which traits are influenced by the same genes. Useful appendices presenting the questionnaires and the intraclass correlations for the twins on each variable are fertile soil for the interested twin researcher. The book can be recommended for its lucid, readable presentation and discussion of the authors' own data and the relevant literature.

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James Shields

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Insect clocks

Insect Clocks (International Series in Pure and Applied Biology. Division. Zoology, Vol. 54.) By D. S. Saunders. Pp. viii+279. (Pergamon: Oxford and New York, July 1976.) £9.25.

THE study of biological clocks is one of those areas of the biosciences that truly straddles the plant and animal kingdoms. The existence of clock-like processes is apparently universal in eukaryotic life, and the clocks of phylogenetically widely separated species exhibit remarkably similar characteristics. Whether this indicates evolutionary convergence or a common, primitive root is not clear, but what it does indicate is why the study of biological clocks has been, and must be multidisciplinary.

The need for a monograph on the clocks of insects may therefore not be immediately apparent. Its justification lies in the simple fact that in no other single order of organisms has so much research been done on so many different kinds of clock. Herein lies the strength of *Insect Clocks*: it provides a comprehensive cover of 'chronobiology' (the subject's American name) in a microcosm.

The book starts off on circadian (that is, 24-h) rhythms, in individuals (chapter 2), in populations (chapter 3), and in physiology (chapter 4). This separation is not entirely happy because rhythms exhibit the same characteristics at whatever level of organisation they occur, so phenomena such as temperature compensation, entrainment and phase-response curves have

to be considered three times over, and the treatment, although thorough, is unnecessarily fragmented.

More than half the book, however, is devoted to insect photoperiodism—that is, the reactions of insects to seasonally changing daylength. Here, the author's touch is sure and his exposition and explanation first rate. Biologists of any specialisation wishing to understand the involutions of this tortuous subject could go a long way before finding a more comprehensive or better account. Whether this otherwise admirable explanation is aided by the chronobiologists' addiction to algebraic jargon is another matter. For example, it is simply irritating, rather than informative, to be told eight times in as many pages (8–16) that τ is the symbol for the free running period of rhythms; and does it help to be given the equation $\tau - T = \Delta\phi$, by way of parenthetical expansion of the perfectly clear phrase "... entrainment is effected by discrete apparently instantaneous phase-shifts ..." (p38)? There is a great deal of such symbolising, much of it seemingly unnecessary.

The author unashamedly sticks to insects, his self-confessed lifelong passion. Although this single-mindedness provides the book with its satisfying unitary structure, it does afflict it with an element of tunnel vision. This has prevented the author from considering any of the recent exciting work on the fundamental mechanisms of circadian oscillators, presumably because that work concerns plants, protista and molluscs. Very little is offered on the mechanisms of oscillators in general (the word 'membrane' does not appear anywhere in the otherwise full index, for example), and even on insect clock mechanisms the coverage is less thorough than in some recent reviews. The reader looking for guidance on the modern ideas about oscillator mechanisms, or for an account of the comparative physiology of clocks will therefore be disappointed.

This is the book's only serious weakness: the unifying thread woven through the fabric of the text is taxonomic rather than physiological. Nevertheless, it is an excellent source work, particularly on photoperiodism, and is to be welcomed for that reason alone. In addition, it provides a much more general textbook on chronobiology than its narrow title implies, a paradigm for the subject as a whole, and in that respect is a considerable advance on any of its rivals with more pretentious titles.

John Brady

John Brady is Reader in Insect Behaviour in the Department of Zoology and Applied Entomology at Imperial College, London, UK.

announcements

Awards

Royal Society Medals:

A Royal Medal to **J. W. Cornforth**, University of Sussex, for his fundamental contribution to our knowledge of the biosynthesis of steroids.

A Royal Medal to **J. L. Gowans**, University of Oxford and Medical Research Council Cellular Immunology Unit, for his distinguished research in the field of immunology, especially as regards the recirculation and immunological role of lymphocytes.

A Royal Medal to **A. Walsh**, Division of chemical physics, Commonwealth Scientific and Industrial Research Organization, Australia, for his distinguished contributions to emission and infra-red spectroscopy and his origination of the atomic absorption method of quantitative analysis.

The Copley Medal to **Dorothy M. C. Hodgkin**, University of Oxford, for her outstanding work on the structure of complex molecules, particularly penicillin, vitamin B₁₂ and insulin.

The Rumford Medal to **I. Prigogine**, professor and director of the Free University of Brussels, for his distinguished contributions to the theory of irreversible thermodynamics

The Davy Medal to **R. E. Richards**, Merton College, Oxford, for his outstanding contributions to nuclear magnetic resonance spectroscopy and its application to chemical and biological problems.

The Darwin Medal to **Charlotte Auerbach**, University of Edinburgh, for her discovery of and continuing work on chemical mutagenesis

The Sylvester Medal to **D. G. Kendall**, University of Cambridge, for his many distinguished contributions to probability theory and its applications.

The Hughes Medal to **S. W. Hawking**, University of Cambridge, for his distinguished contributions to the application of general relativity to astrophysics, especially to the behaviour of highly condensed matter.

Meetings

December 14–16, **Biochemical Society**, 566th Meeting, Cambridge; January 6–7, 1977, 577th Meeting, Durham (Meetings Officer, 7 Warwick Court, Holborn, London WC1).

January 4–5, 1977, **Terpenoid Biochemistry**, London (Dr M. F. Roberts, School of Pharmacy, London Univer-

Person to Person

An appeal has been launched for £40,000 to commemorate Dr Henry Miller, Professor of Neurology and Vice-Chancellor of Newcastle University, who died August 25, 1976. It is planned to found a research post in the Department of Neurology and to give an annual concert or recital. Donations and enquiries to Mr A. Tindall, 12 Windsor Terrace, Newcastle upon Tyne, UK.

Chemist and wife have furnished house in Cambridge: three large bedrooms, well fitted kitchen, gas-fired central heating, garage, quiet location, convenient for University departments, MRC units, Addenbrookes hospital. Wish to exchange for furnished house in or near Williamsburg, Virginia while on sabbatical leave, August 1977–August 1978. Peter Sykes, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.

There will be no charge for this service. Send items (not more than 60 words) to Marcus Dobbs at the London Office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

sity, 29/39 Brunswick Square, London WC1).

January 12, **Dielectrics**, London (Meetings Officer, Institute of Physics, 47 Belgrave Square, London SW1).

January 16, **Radiation and Materials Modification**, London (address as above).

March 23–25, **Radiation Effects in Liquids and Solids**, Leicester (Mrs Y. A. Fish, Chemical Society, Burlington House, London W1).

March 29–31, **Anisotropic Dielectrics**, Cambridge (Dr C. W. Smith, Department of Electrical Engineering, University of Salford).

March 27–April 8, **Electron Microscopy in Biology**, Erice, Sicily (Dr I. R. Pasquali, Istituto di Patologia Generale, Via Campi 287, Modena, Italy).

April 4–6, **Biochemical Aspects of Plant and Animal Coevolution**, Reading (J. B. Harborne, Department of Botany, University of Reading).

May 30–June 3, **Ferritic Steels for Fast Reactor Steam Generators**, London

(Institution of Civil Engineers, 1–7 Great George Street, London SW1).

June 7–9, **Trace Substances in Environmental Health**, Columbia, Missouri (Dr D. D. Hemphill, 411 Clark Hall, University of Missouri, Columbia, Missouri 65201).

June 20–22, **Mass Spectroscopy in Biochemistry and Medicine**, Riva del Garda, Italy (Dr A. Frigerio, Istituto de Ricerche Farmacologiche, Via Eritrea, 62, Milan, Italy).

July 11–18, **Physical Chemistry and Hydrodynamics** (Levich 60th Birthday Conference), Oxford (D. B. Spalding, Imperial College, Exhibition Road, London SW7).

July 25–29, **High Pressure Applications and Techniques**, Boulder, Colorado (K. D. Timmerhaus, Engineering Research Center, University of Colorado, Boulder, Colorado 80302).

July 26–28, **Synthesis in Organic Chemistry**, Oxford (J. F. Gibson, The Chemical Society, Burlington House, London W1).

September 5–8, **Acetylenes, Allenes and Cumulenes**, Nottingham (address as above).

September 19–23, **Biological Oxidation of Nitrogen in Organic Molecules**, London (J. W. Gorrod, Chelsea College, 271 King Street, London W6).

September 26–29, **Heterogeneous Oxidation**, Leeds (J. F. Gibson, Chemical Society, Burlington House, London W1).

October 24–28, **Water Chemistry of Nuclear Reactor Systems**, Bournemouth, England (Institution of Civil Engineers, 1–7 Great George Street, London SW1).

November 14–17, **Pineal Organ**, Jerusalem (I. Nir, Faculty of Medicine, Hadassah Medical School, Jerusalem).

November 28–December 1, **Optimisation of Sodium-cooled Fast Reactors**, London (Institution of Civil Engineers, 1–7 Great George Street, London SW1).

December 12–14, **Monitoring of Hazardous Gases in the Working Environment**, London (J. F. Gibson, Chemical Society, Burlington House, London W1).

May 13–19, 1979, **Radiation Research**, Tokyo (S. Okada, Department of Radiation Biophysics, Faculty of Medicine, University of Tokyo, Hongo, Tokyo, Japan).

Recent scientific and technical books

Physics

FULLER, Everett G., and HAYWARD, Evans (edited by). *Photonuclear Reactions* (Benchmark Papers in Nuclear Physics/2). Pp.xviii+426. ISBN-0-470-15142-0. (Stroudsburg, Pennsylvania: Dowden, Hutchinson and Ross, Inc., 1976. Distributed by Halsted Press, a Division of John Wiley and Sons, Inc., New York and London.) \$38; £21.

KILLEEN, John. *Controlled Fusion*. (Methods in Computational Physics: Advances in Research and Applications, Vol. 16.) Pp.xiii+450. ISBN-0-12-460816-7. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1976.) \$47; £27.25.

KESSLER, Joachim. *Polarized Electrons* (Texts and Monographs in Physics). Pp.ix+223. ISBN-3-540-07678-6. (Berlin and New York: Springer-Verlag, 1976) DM 59.80; \$24.60.

LANDOLT-BÖRNSTEIN, Numerical Data and Functional Relationships in Science and Technology/Zahlenwerte und Funktionen aus Naturwissenschaften und Technik. New Series/Neue Serie. Editor-in-Chief. K. H. Hellwege. Group 2: Atomic and Molecular Physics/Atom- und Molekularphysik. Vol. 7. Structure Data of Free Polyatomic Molecules/Strukturdaten freier Mehratomiger Moleküle. By J. H. Callomon, E. Hirota, K. Kuchitsu, W. J. Lafferty, A. G. Maki and C. S. Pote. With the assistance of I. Buck and S. Starck. Edited by K. H. Hellwege and A. M. Hellwege. Pp.vii+395. ISBN-3-540-07577-1. (Berlin and New York: Springer-Verlag, 1976) DM 360; \$147.60.

LANDOLT-BÖRNSTEIN, Numerical Data and Functional Relationships in Science and Technology/Zahlenwerte und Funktionen aus Naturwissenschaften und Technik. New Series/Neue Serie. Editor-in-Chief. K. H. Hellwege. Group 2: Atomic and Molecular Physics/Atom- und Molekularphysik. Vol. 8 (Supplement to Volume 7/Ergänzung zu Band 7. Magnetic Properties of Coordination and Organometallic Transition Metal Compounds/Magnetische Eigenschaften der Koordinations- und Metallorganischen Verbindungen der Übergangselemente, Ergänzungsband 1 (1964-1968). Editors: K. H. Hellwege and A. M. Hellwege. Pp.xviii+1163. ISBN-3-540-07441-4. (Berlin and New York: Springer-Verlag, 1976.) DM 1100; \$451.

MEHRING, M. *High Resolution NMR Spectroscopy in Solids*. (NMR: Basic Principles and Progress/Grundlagen und Fortschritte, Vol. 11.) Pp.xi+246. ISBN-3-540-07704-9. (Berlin and New York: Springer-Verlag, 1976) DM 68; \$27.90.

Chemistry

FULLER, C. W. (edited by). *Annual Report on Analytical Atomic Spectroscopy: Reviewing 1975*, Vol. 5. Pp.viii+267. ISBN-0-85186-757-X. (London: The Chemical Society, 1976.) £15; \$33.75.

HEILBRONNER, Edgar, and BOCK, Hans. *The HMO Model and its Application*. Vol. 2. *Problems with Solutions*. Translated by William Martin and Anthony J. Rackstraw. Pp.viii+449. ISBN-0-471-01473-7. (London and New York: Wiley-Interscience, John Wiley and Sons; Weinheim: Verlag Chemie, GmbH, 1976.) £15; \$33.00.

HEILBRONNER, Edgar, and BOCK, Hans. *The HMO Model and its Application*. Vol. 3. *Tables of Huckel Molecular Orbitals*. Translated by William Martin and Anthony J. Rackstraw. Pp.190. ISBN-0-471-01474-5. (London and New York: John Wiley and Sons, Weinheim: Verlag Chemie, GmbH, 1976.) £9; \$18.

KEITH, Lawrence H. (edited by). *Identification and Analysis of Organic Pollutants in Water*. Pp.x+718. ISBN-0-250-40131-2. (Ann Arbor, Michigan: Ann Arbor Science Publishers, Inc.; Chichester: John Wiley and Sons, Ltd., 1976) \$30.25; £17.90.

PEARSON, Ralph G. *Symmetry Rules for Chemical Reactions: Orbital Topology and Elementary Processes*. Pp.ix+548. ISBN-0-471-01495-8. (New York and London: Wiley-Interscience, John Wiley and Sons, 1976.) \$31; £17.50.

Technology

BLASCHKE, W. S., and MCGILL, J. *The Control of Industrial Processes by Digital Techniques: The Organization, Design and Construction of Digital Control Systems*. Pp.viii+185. ISBN-0-444-41493-2. (Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company, 1976.) Dfl. 77; \$29.75.

EVAN-IWANOWSKI, R. M. *Resonance Oscillations in Mechanical Systems*. Pp.xi+292. ISBN-0-444-41474-6. (Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company, 1976.) Dfl. 77; \$29.75.

FLOWERS, T. H. *Introduction to Exchange Systems*. Pp.xvii+326. ISBN-0-471-01865-1. (London and New York: Wiley-Interscience, John Wiley and Sons, 1976.) £13; \$26.

GOULARD, R. (edited by). *Combustion Measurements: Modern Techniques and Instrumentation*. (A Project Squid Workshop) Pp.xii+483. ISBN-0-12-294150-0. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers; Washington and London: Hemisphere Publishing Corporation, 1976.) \$34.50; £21.05.

JACOBSON, Kurt I., and JACOBSON, Ralph E. *Imaging Systems: Mechanisms and Applications of Established and New Photosensitive Processes*. Pp.319. (London and New York: The Focal Press, 1976) £11.95 net.

MADDIN, Robert (editor-in-chief). *Challenges and Opportunities in Materials Science and Engineering*. (Anniversary volume of *Materials Science and Engineering*.) Pp.vi+264. (Lausanne: Elsevier Sequoia, S. A., 1976.)

MEDELSSOHN, K. (edited by). *Proceedings of the Sixth International Cryogenic Engineering Conference*, Grenoble, 11-14 May 1976. (ICEC, 6) Pp.827. ISBN-0-902852-58-2. (Guildford: IPC Science and Technology Press, Ltd., 1976.) £29.

McDOUGALL, Angus. *Fuel Cells* (Energy Alternatives Series) Pp.xii+147. ISBN-333-18408-4. (London and Basingstoke: The Macmillan Press, Ltd., 1976) Hard cover £7.95; Paper cover £2.95.

PALMER, Andrew C. *Structural Mechanics* (Oxford Engineering Science Texts.) Pp.x+218. ISBN-0-19-856128-8. (Oxford: Clarendon Press; London: Oxford University Press, 1976) Paper £3.50 net; Boards £7 net.

ROBERTSON, J. Craig. *A Guide to Radiation Protection*. Pp.86. ISBN-333-19278-8. (London and Basingstoke: The Macmillan Press, Ltd., 1976.) £4.95.

ROBERTSON, Angus (edited by). *Video Yearbook 1977*. Pp.286. ISBN-0-85642-064-6. (Poole: Dolphin Press, an imprint of Blandford Press, 1976) £4.75.

RUSSELL, Clifford S., and VAUGHAN, William J. *Steel Production: Processes, Products, and Residuals*. Pp.xx+328. ISBN-0-8018-1824-9. (Baltimore and London: The Johns Hopkins University Press, 1976. Published for Resources for the Future.) £12.40.

SZEKELY, Julian, EVANS, James W., and SOHN, Hong Yong. *Gas-Solid Reactions*. Pp.xiii+400. ISBN-0-12-680850-3. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1976.) \$39.50, £24.10.

Earth sciences

GOLDBERG, Edward D. *Strategies for Marine Pollution Monitoring*. Pp.x+310. ISBN-0-471-31070-0. (New York and London: Wiley-Interscience, John Wiley and Sons, 1976.) \$27.50; £16.50.

Biological sciences

ANDERSON, J. M., and MADFADYEN, A. (edited by). *The Role of Terrestrial and Aquatic Organisms in Decomposition Processes* (The 17th Symposium of The British Ecological Society, 15-18 April 1975.) Pp.xi+474. ISBN-0-632-00018-X. (Oxford and London: Blackwell Scientific Publications, 1976.) £14.

BAKER, Joseph T., and MURPHY, Vreni. *Handbook of Marine Science* Vol. 1: *Compounds from Marine Organisms*. Pp.228. ISBN-0-87819-388-X. (Cleveland, Ohio: CRC Press, Inc., 1976.) \$29.95.

BALTIMORE, David, HUANG, Alice S., and FOX, C. Fred (edited by). *Animal Virology* (ICB-UCLA Symposium on Molecular and Cellular Biology, Vol. IV, 1976.) Pp.xi+824. ISBN-0-12077350-3. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1976.) \$29.50; £18.

BANKS, P., BARTLEY, W., and BIRT, L. M. *The Biochemistry of the Tissues*. Second edition. Pp.xv+493. ISBN-0-471-05471-2. (London and New York: John Wiley and Sons, 1976.) Cloth £14.50; \$30. Paper £6.26; \$13.50.

BROWN, Leslie. *Eagles of the World*. Pp.224. ISBN-0-7153-72696. (Newton Abbot and London: David and Charles, 1976.) £4.95.

BROWN, Leslie. *Birds of Prey: Their Biology and Ecology*. Pp.256. ISBN-0-600-31306-9. (London and New York: Hamlyn Publishing Group, Ltd., 1976.) £4.50.

CAPUTO, A. (edited by). *Biological Basis of Clinical Effect of Bleomycin*. (Progress in Biochemical Pharmacology, Vol. 11.) Pp.x+230. ISBN-3-8055-2338-6. (Basel, London and New York: S. Karger, 1976.) Sfr./DM 127.

CHAPMAN, V. J. *Coastal Vegetation* Second edition. (Pergamon International Library of Science, Technology, Engineering and Social Studies.) Pp.viii+292. ISBN-0-08-020896-7. (Oxford and New York: Pergamon Press, 1976.) Hard case £8.75, Flexicover £4.75.

History of science

BRIEGER, Gert H. (edited by). *Theory and Practice in American Medicine: Historical Studies from the Journal of the History of Medicine and Allied Sciences*. Pp.xv+272. (New York: Science History Publications, a Division of Neale Watson Academic Publications, Inc., 1976.) Cloth \$12, Paper \$4.95.

COLLARD, Patrick. *The Development of Microbiology*. Pp.201. ISBN-0-521-21177-8. (Cambridge and London: Cambridge University Press, 1976.) £6.50.

COPERNICUS. *On the Revolutions of the Heavenly Spheres*. A New Translation from the Latin with an introduction and notes by A. M. Duncan. Pp.328. ISBN-0-7153-6927-X. (Newton Abbot and London: David and Charles; New York: Barnes and Noble Books, a Division of Harper and Row, Publishers, 1976.) £12.50.

GIFFORD, Jr., George E. (edited by). *Physician Signers of the Declaration of Independence*. Pp.163. ISBN-0-88202-159-1. (New York: Science History Publications, a Division of Neale Watson Academic Publications, Inc., 1976) \$10.

KANGRO, Hans. *Early History of Planck's Radiation Law*. Pp.xvii+282. ISBN-0-85066-063-7. (London: Taylor and Francis, Ltd., 1976.) £25.

McCORMACH, Russell (edited by). *Historical Studies in the Physical Sciences*. Seventh Annual Volume. Pp.xciv+489. ISBN-0-691-08169-7. (Princeton, NJ: Princeton University Press, 1976.) £19.80.

Anthropology

BAKER, Paul T., and LITTLE, Michael A. (edited by). *Man in the Andes: a Multidisciplinary Study of High-Altitude Quechua*. (US/IBP Synthesis Series 1.) Pp.xx+482. ISBN-0-87933-228-X. (Stroudsburg, Pennsylvania: Dowden, Hutchinson and Ross, Inc., 1976. Distributed by Halsted Press, a Division of John Wiley and Sons, Inc., New York and London.) £18; \$30.

Psychology

BARBER, T. X. *Pitfalls in Human Research: Ten Pivotal Points*. (Pergamon General Psychology Series.) Pp.vii+117. ISBN-0-08-020935-1. (Oxford and New York: Pergamon Press, 1976.) \$6.95.

JUNG, C. G. *Analytical Psychology: Its Theory and Practice*. (The Tavistock Lectures.) Pp.xvi+224. ISBN-0-7100-8414-5. (London and Henley: Routledge and Kegan Paul, Ltd., 1976.) Paperback £1.95.

LIPSITT, Lewis P. (edited by). *Developmental Psychobiology: The Significance of Infancy*. Pp.x+143. ISBN-0-470-15127-7. (Hillsdale, NJ: Lawrence Erlbaum Associates, Publishers, 1976. Distributed by Halsted Press, a Division of John Wiley and Sons, New York and London.) \$12.65, £7.65.

MEDLIN, Douglas L., ROBERTS, William A., and DAVIS, Roger T. (edited by). *Processes of Animal Memory*. Pp.xi+267. ISBN-0-470-15189-7. (Hillsdale, NJ: Lawrence Erlbaum Associates, Publishers, 1976. Distributed by the Halsted Press Division of John Wiley and Sons, New York and London.) \$19, £11.50.

NORMAN, Donald A. *Memory and Attention: An Introduction to Human Information Processing*. (Series in Psychology.) Second edition. Pp.xlii+262. ISBN-0-471-65136-2. (New York and London: John Wiley and Sons, Inc., 1976.)

PORGES, Stephen W., and COLES, Michael G. H. (edited by). *Psychophysiology* (Benchmark Papers in Animal Behavior/6.) Pp.xv+366. ISBN-0-470-15136-6. (Stroudsburg, Pennsylvania: Dowden, Hutchinson and Ross, Inc., 1976. Distributed by Halsted Press, a Division of John Wiley and Sons, Inc., New York and London.) \$32; £17.75.

Sociology

BLACK, Donald. *The Behavior of Law*. Pp.xi+175. ISBN-0-12-102650-7. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1976.) \$12.50; £7.60.

CHIROI, Daniel. *Social Change in a Peripheral Society: The Creation of a Balkan Colony*. (Studies in Social Discontinuity.) Pp.xvii+179. ISBN-0-12-173150-2. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1976.) \$12.50, £7.25.

KORNITZER, Margaret. *Adoption*. Pp.186. Fifth edition. ISBN-0-370-10059-X. (London: Putnam and Company, 1976.) Hardback £4.95, Paperback £3.50.

McKEOWN, Thomas. *The Modern Rise of Population*. Pp.168. ISBN-0-7131-5867-0. (London: Edward Arnold (Publishers), Ltd., 1976) £7.95.

ROSE, Hilary, and ROSE, Steven. *The Radicalization of Science: Ideology of the Natural Sciences*. (Critical Social Studies.) Pp.xxvi+205. ISBN-0-333-21141-3. (London and Basingstoke: The Macmillan Press, Ltd., 1976.) Hard cover £10; Paper cover £3.95.

ROSE, Hilary, and ROSE, Steven (edited by). *The Political Ecology of Science: Ideology of the Natural Sciences*. (Critical Social Studies.) Pp.xxvi+218. ISBN-0-333-21139-1. (London and Basingstoke: The Macmillan Press, Ltd., 1976.) Hard cover £10, Paper cover £3.95.

Geography

OEHSER, Paul H. (compiled and edited by). *Research Reports*. (Abstracts and reviews of research and exploration authorized under grants from the National Geographic Society during the year 1968.) Pp.x+527. ISBN-087044-136-1. (Washington, DC: National Geographic Society, 1976.) \$5.

General

BUSVINE, J. R. *Insects, Hygiene and History*. (London: The Athlone Press, University of London, 1976. Distributed by Tiptree Book Services, Ltd., Tiptree, Essex; Humanities Press, Inc., New Jersey.) £6.95.

FARSON, Daniel (compiled by). *In Praise of Dogs*. Pp.118. (London: George G. Harrap and Co., Ltd., 1976) £3.95 net.

GOLDSTEIN-JACKSON, Kevin. *Experiments With Everyday Objects*. Pp.187. ISBN-0-285-62245-5. (London: Souvenir Press, Ltd., 1976) £3.

ROSZAK, Theodore. *Unfinished Animal: The Aquarian Frontier and the Evolution of Consciousness*. Pp.ix+271. ISBN-0-571-11014-2. (London: Faber and Faber, Ltd., 1976.) £2.95.

nature

December 9, 1976

Some seasonal greetings

To our leader writer:

May all your issues be central ones; may every corrective measure be at once too little and too much.

To book blurb writers:

May all your wares continue to be of interest to highly qualified specialists, students and the layman alike.

To those eminences who require their secretary to type after their name MA, PhD, ScD, FRIC, FInstP, FICE, CEng, FRSEdin:

May you excuse the modesty of our response.

To Hills Road, Cambridge:

May your access to our columns be as easy as everyone else in the world assumes it is.

To all our London subscribers:

May we never again send you *Nursing Times* instead of *Nature*. Think of what the poor nurses had to wade through.

To all directors of big government laboratories:

May you never again need to be thanked for granting permission to your staff to publish.

To all those whose notepaper is headed:

US Department of Health, Education and Welfare,
Federal Ventures Office,
Bureau of Diseases,
Common Cold Eradication Program,
Viral Infection Project,
University of South Chicago,
Western Campus,
Emily Smoot Building,
Department of Biochemistry,
Sub-department of Sneeze Research,
Room 3581

May you get a box number.

To all authors of rejected papers:

Even though you are 'naturally disappointed' by our decision, may you maintain your custom of 'not being in the habit of writing to complain'.

To all those who telephone us and find us unavailable:

May you ring us again; we won't ring you.

To all our readers:

May your grumbles find their way to us; we may not be on the same grapevine as you.

To a scientific jet-setter:

May you look on the messages board at Heathrow airport. We don't send letters to you at your department any more.

To the editors of new journals:

May there always be interdisciplinary subjects crying out for a new journal dedicated to rapid dissemination of results; may you find a goodly number of Nobel Laureates for your editorial board.

To our referees:

May we never again send an expert on tadpoles a paper on general relativity. And if we do may it never take a month for him to let us know.

To Tom Jukes and Kenneth Mellanby:

May you find issues for your columns as black and white as we make your faces.

To our printers:

May we come to some agreement on the spelling of millennium.

To Professor J. L. Jinks:

May we never again call you Professor J. L. Links.

To our sub-editors:

May you remember the difference between partly and partially, and between compared with and compared to; may you put b.p. but bc.

To readers of News and Views:

May you find your own work described therein—and not in slighting tones either.

To our authors:

May you put your conclusion in your first paragraph. After that can come the introduction. The middle bit then goes at the end.



The Flowers Report: opportunities missed

Dr R. H. Mole, Director of the MRC Radiobiology Unit at Harwell, offers some personal reflections on the recent nuclear power report of the Royal Commission on Environmental Pollution

THE Report's main conclusion is that there are substantial environmental objections to a nuclear power programme on the scale envisaged (paragraph 534) and that we should not rely for energy supply on a process that produces such a hazardous substance as plutonium unless there is no reasonable alternative (paragraph 535). In an earlier paragraph (186), the conclusion is much more categorical: although aware of the advantages that nuclear power offers the Commission did not think that these outweigh the risks described. Routine electricity production by nuclear means, however, appears to be regarded as unobjectionable.

It is necessary to consider important omissions from the Report, so important as to appear to warrant asking whether these conclusions are justified.

The human cost

The routine environmental effects of nuclear power may well be much less damaging than those of fossil fuelled power stations... The discharges of radioactivity from nuclear stations, both to air and water, are radiographically insignificant and there are no discharges of offensive gases or particulates. (191).

... the aspects of nuclear development which cause most dissension tend to be those that relate not to normal operation but to the possibility of abnormal events such as accidents at nuclear installations or malevolent acts against them. (10).

Although the Report says that the Commission "should be concerned not so much with the individual risk as with the total effect of possible accidents" (173) this total effect is never considered. The human cost of nuclear power is considered and enumerated only in relation to single examples of what were said to be very unlikely events, an exceptional release of radioactivity into the sea at Windscale (139) or into the atmosphere from a serious reactor accident (263-268). There is nothing in the Report to show that the Commission considered or enumerated the total casualty rate from reactor malfunctions and small accidents which will occur much more frequently than exceptional severe accidents, or even from the routine operation of power reactors without malfunction. Is it a mistake to conclude that, when the Commission concentrated on exceptional occurrences, it did not know whether it had addressed itself to the real problem of nuclear safety or only to a minor aspect of it?

In relation to the possibility of terrorist activities the Commission con-

tented itself with stressing that purloining enough plutonium to make a crude bomb is bound to happen sooner or later but did not attempt to deduce the likely or possible consequences in terms of numbers of casualties. Thus on this question the Royal Commission with all its expertise and resources has added nothing to what has been said repeatedly over the past few years by the ordinary journalist.

Energy Strategy

Chapter IX on Energy Strategy considers energy needs and alternative means of satisfying those needs but nowhere considers the human costs of getting energy by any means, except to take for granted that nuclear power is basically hazardous. Much of the Report's anxiety seems to be based on "the assumed necessity of securing steadily increasing energy supplies" (197) as illustrated in Table 14. But no facts are provided to enable the reader to consider what methods of supplying energy might be the safest to the population and the least damaging to the environment if there was no need to increase energy supplies at all. Chapter VI is said to relate nuclear and non-nuclear hazards (16) but it does not do this for alternative means of getting energy.

The Commission's failure to compare the human costs of alternative sources of energy and to consider the total effect of possible accidents can make its anxieties about exceptional serious accidents seem wholly unbalanced. This can be illustrated as follows. The current death rate from accidents amongst employees of the National Coal Board is in round terms 50-60 per year. The number of cancer deaths from a particular example of failure of containment of Caesium-137 at Windscale is given as perhaps fifty in total (spread out over many years). Such an event is said not to be at all likely and its consequences are used to illustrate the need for the strictest containment of radioactive wastes for very long periods (139), as if the number of casualties was something out of the ordinary and perhaps unacceptable to the Commission.

The large radioactivity release considered as an exceptional but very serious event is of 10^7 Ci Iodine-131, 10^6 Ci Caesium-137 and so on, and would give 110-350 deaths from cancer (263-268). This is the same as the number of accidental deaths in coal production in UK over a 2-6 year period

at the current annual rate. The probability of release of 10^7 Ci Iodine-131 is 10^{-7} per reactor year (the Report's Figure 15). One hundred reactors each of 1,000 MW would produce twice as much electricity as the whole of that generated by all means in UK in 1975 (462, 468), and the probability of release of 10^7 Ci Iodine-131 would then be once every 100,000 years. On this count alone nuclear power would appear to be more than 10,000 times safer than coal power.

Such a comparison is obviously inadequate since it is between a most exceptional event and a routine annual occurrence. It shows very clearly how misleading it may be to concentrate on exceptional events with large consequences and to ignore much commoner events each with relatively small consequences. Differences in emphasis on these contrasting sources of risk were in fact noted by the Royal Commission as the reason for inconsistencies in evidence on the policy for siting reactors (296), but the Commission seems to have failed to appreciate the significance of the matter for itself.

Consistency in assessing the acceptability of risk

The Royal Commission emphasised the need for a body with statutory responsibility for endorsing the basic standards for radiological protection on the national behalf (223). Such a body must be clearly independent of any interests promoting developments involving radioactivity, it is agreed, but the conclusion can be questioned that "in view of the importance of the issues it should be a body constituted specifically for radiological protection" (223). Whatever the history of how the basic standards were chosen, they are expressed in terms of tissue dose and, therefore, can be specifically related to risk.

Technically this relation must be as exact as current scientific understanding will allow, but "the importance of the issues" at the present time is not so much the degree of technical accuracy as the acceptability of the corresponding risk. This cannot be wholly determined by any body specifically constituted only for radiological protection, as that phrase would normally be understood, because acceptability is not a radiological matter. The non-technical essence of the choice of basic standard is to set the corresponding level of risk at that point on the scale of risk which makes it acceptable in the light of all other sources of everyday risk in work and generally.

The Royal Commission recognised that the collective action of involved government departments was inad-

equate for providing advice on energy strategy (515) and recommended the need for an independent high-level advisory body on the subject (535). All the more reason for recognising the need for a central body to consider and report publicly on the level of acceptability of risk in relation to the various means by which the great variety of national needs could be satisfied, energy supply being but one of these. Consistency in assessing acceptability of risk is required not only within the energy field but also between the energy field and other major national activities not excluding the practice of medicine.

For example, at the present time roughly as much Iodine-131 is administered to patients, and from them is released into the environment each year, as would be released on average per year over the 100,000-year period within which one most exceptional release of 10^7 -Ci Iodine-131 might be expected from a hundred nuclear reactors routinely generating electricity.

Public reaction to risks

A major factor in the Royal Commission's approach, although not made wholly explicit, is expressed clearly enough in the Report: risks of events that affect people individually or in small numbers, it says, "are fairly readily accepted as inevitable", but events "that cause death or injury to many people at one time . . . have, in proportion to the numbers affected, a much greater impact in creating public concern" (172 and see 270).

The Commission, in emphasising so strongly the exceptional event and accepting almost without question the safety of routine discharges of radioactivity into the environment, seems to have adopted the same attitude. Even if the members of the Royal Commission regarded themselves as in some sense representatives of the public or trustees of the national interest, there seems no reason why they should accept these public attitudes as given and unchangeable and appropriate for themselves. It was surely within their remit to attempt to change public attitudes by explanation and reason. This would certainly have to be a function of a central body to consider acceptability of risk, as suggested above, if it was to succeed in providing consistent guidelines on emotive subjects.

Responsibilities and experts

The Royal Commission found it difficult to obtain independent but expert advice "since the acknowledged experts are often themselves involved in the related developments" (163). So far as assessing the effect of pollutants, including radioactivity, "we can do no better than require that the agencies concerned are expert, open and in-

dependent, and such as to ensure that necessary research is undertaken and that assessments are appropriately revised in the light of new knowledge. The requirement reflects on organisational arrangements" which are required (188).

The International Commission on Radiological Protection (ICRP) "is only as good as its members, and it is vitally important that these should continue to be appointed independently of the approval of their national governments and purely on the basis of their professional standing amongst their scientific peers . . . We hope that there will continue to be a strong British representation on the ICRP . . ." (203).

A main problem, however, with the Commission's recommendations for systematising responsibilities is that they will still further reduce the possibilities of finding informed and at the same time independent experts, that is independent of interested organisations.

Bodies with statutory responsibilities as proposed by the Commission, such as the strengthened NRPB, the Nuclear Waste Management Committee, the Nuclear Waste Disposal Corporation, the strengthened Health and Safety Executive (531) and HMPI (527), will clearly attract to themselves the best experts they can find. Any British members of ICRP will inevitably be found to belong to NRPB as, even now, three out of four current British members of ICRP do. Each such body will get financial support from government sources. Are they in the least likely to criticise each other publicly as the Royal Commission said they should be free to do? If an organisation has statutory responsibilities it must have a "party line" to which its employees conform. Even if, like NRPB at present, it is a national centre for advice without statutory responsibilities, it must ensure consistency in the advice it gives. Is this conducive to criticism from within of scientific or technological bases of action?

What the Royal Commission quite rightly seemed to desire was to maximise the possibilities for what might be called informed assent and dissent. This requires not only publicly available information but the existence of individuals who are not beholden to organisations with statutory responsibilities and who are technically educated and intellectually free to pursue argument and the advance of knowledge without the confinement of terms of reference or administrative boundaries.

Is there a big enough reservoir of such individuals for national needs? It wouldn't seem so for radiobiologists if major reports in Britain, like the Royal Commission's, and in the USA, like the

Rasmussen Report on Reactor Safety, were each radiobiologically defective, and the same reasoning can be applied to any other scientific field covered by the Royal Commission Report. The only way of remedying this sort of deficiency is to see that universities and polytechnics pay sufficient attention to the field of knowledge and that research councils play their part.

Research priorities

The Royal Commission regarded the arrangements for initiation and co-ordination of research as of the greatest importance but in no case was the priority of research discussed in relation to the quantitative importance of the subject matter as it relates to risk. Research could well be justified if a more than negligible risk is to be assessed but, if routine nuclear power is already known to be unobjectionable as suggested by the Commission, would research on its hazards be needed? It could hardly be justified merely by legislative requirements for annual surveys of discharges of radioactivity (251).

Debate and accessibility of fact

In its last few paragraphs the Royal Commission Report quite rightly stressed the importance of public understanding of the long term issues of unusual range and difficulty raised by nuclear development (521), and the need for open and deliberate weighing of risks and costs of embarking on a major nuclear programme against those of not doing so (522). But such an open political process will generate only hot air and useless emotion unless there is an agreed basis of technological fact from which to start. The scientific convention is to provide a reference for every possibly arguable fact, and this systematic buttressing by checkable publications is something the dyed-in-the-wool scientific reader rather sadly misses. The enquirer cannot make the checks he may desire: for example, the source of only ten of 25 Figures and three of 14 Tables is acknowledged, and even then a specific reference is provided for only four of the 13 sources acknowledged. Perhaps a Royal Commission Report, especially if it deals with complex issues, is not a suitable place for a comprehensive reference list, but it must be a retreat from established scientific methodology if a Report of a Commission on highly technical questions is without adequate references because then its authority is simply the eminence of its individual members. This is a special problem in the present case, because many readers of different backgrounds have recognised serious defects in the Report.

For instance, the Royal Commission

was concerned that insufficient research and attention had been given to atmospheric pollution by radioactivity (234), yet there is no evidence that the Commission knew of the more than 100 scientific papers on this subject published from the Environmental Sciences and Medical Division of AERE.

Conclusions

The Report of the Royal Commission on Environmental Pollution fails to provide (i) any evaluation of the relative level of risk of nuclear power from accidents and from normal operations, (ii) any corresponding evaluation of

risk from other means of energy supply, (iii) any recommendation for a means of considering the level of risk in relation to acceptability on the national behalf.

Without (i) it would seem that the Royal Commission cannot know whether or not, in its emphasis on exceptional occurrences, it has tackled the real problem. Without (ii) its examination of Energy Strategy is woefully inadequate. Without (iii) its recommendation for a strongly based national body to consider radiological protection leaves that body with no standards by which to take decisions.

The Report concluded that plutonium must not be produced on a large scale until it is demonstrated that the plutonium can be taken care of safely. This is bound to be generally agreed. But safety is an open-ended concept and it must be a matter of judgment to decide whether what is known does or does not amount to the demonstration of a sufficient degree of safety. No criteria are provided by which such a judgment could be made.

Without all these, how can there be a deliberate weighing of the risks, costs and advantages of a major nuclear programme? □

Flowers on Flowers

Sir Brian Flowers, who recently stepped down as Chairman of the Royal Commission after 3½ years, made his first public comments on reaction to the nuclear power report when he spoke at the British Nuclear Energy Society last week. Some extracts:

On the disposal of radioactive waste: "I have occasionally been asked what we meant by demonstrating a method beyond reasonable doubt. There appears to be agreement that a full programme of research might take a decade or more, but we argue that in any case we have that time available before becoming irretrievably committed to nuclear power as a major component of our electricity supply. I would suggest that what is needed in addition to a vigorous, systematic research programme is to choose and to set aside as soon as possible at least one specific site for which there is a consensus of geological, hydrological and other relevant opinion that it is acceptable for inaccessible disposal. It need not be the one eventually adopted, but it would provide a fall-back position. This, too, is a problem that exists irrespective of the future of nuclear power."

On alternative energy strategies: "The crucial long-term issues are on the one hand the competition between nuclear energy and coal, and on the other hand between both and renewable resources; and also between high electricity and therefore high heat-waste, on the one hand, and vigorous conservation measures on the other. Whether it is looked at from the industrial or the environmental point of view there is a very strong element of nuclear versus the rest. In these circumstances it is vital that responsible advice to Ministers should be seen to be independent of the major factions in the argument. I believe that it should be a matter of public

concern that this is not at present the case."

On the possible effects of illicit activities: "On this issue, alone amongst all those we studied, we were given very little help by official bodies . . .

" . . . Probably the Government already has all the legal powers it would need under the Anti-Terrorist Acts of 1974 and 1976. Is Mr Eadie [Minister of State at the Department of Energy] saying that the Government has consciously resolved that the use of these powers is acceptable to safeguard the future electrical power industry? And that the changes then implicit for our society will be amply offset by the economic benefits of nuclear power? Perhaps he is, and perhaps he is right; but how are we to know unless we can discuss the matter? The Royal Commission does not know the answers to these questions. It merely considers them important and asks that they be discussed before we develop a reliance on nuclear power. If the Government continues to skulk behind the Official Secrets Act we may not know until it is too late to wish that it were otherwise."

On nuclear weapons proliferation: " . . . Thanks to the vested interests of the nuclear weapons states; thanks to the huge R&D resources already devoted to the development of nuclear power; thanks to the almost universal belief in a continuing abundance of cheap electricity, only recently shown to be a myth by the oil sheiks; thanks to the ensuing lack of attention paid to the need for energy conservation, the exploration of more meaningful long-term energy strategies, and the development of alternative sources; and on the global scale, thanks above all to the lack of any effective population control—it is simply not possible in 1976 to state with the required

degree of certainty that the world need not be dependent on nuclear power beyond the end of this century. A severe lack of energy, and the continued division of the world into rich and poor which some would then envisage, might itself be extremely dangerous, even to the extent of bringing in the very nuclear hazards we were seeking to understand and if possible to lessen."

On the question of developing a commercial fast breeder reactor:

"Judging by experience from other prototype reactors we shall still be learning a great deal from it in five or even ten years' time. In any case, it is now unrealistic to suppose that the first full-scale breeder reactor, the so-called CFR-1, could be started in under three or four years, even given the approval tomorrow. That gives ample time for the nuclear debate to come to a conclusion. I believe one should contemplate building any such reactor on a remote site contiguous with its own recycling plant so that there would be no need, after the first loading, for substantial quantities of plutonium to enter or leave the site. It is hardly likely that the result would justify the adjective 'commercial', but I believe it could be done in an environmentally acceptable fashion provided there is no commitment to an ongoing programme."

"But I see no need for undue speed, even if one believes there is no avoiding fast reactors and the plutonium economy they entail. Thermal reactor development has proved to be within the capacity of the United Kingdom, but only just. Fast reactor development is likely to be beyond our resources. It seems to me essential that if we pursue it at all we should do so on a European basis. I can see no reason for wishing it were otherwise now that we are fully committed to the European Community . . ."

Nature's Christmas issue a year ago carried an article entitled 'Naming the Loch Ness monster'. This year, **Carl Sagan** writes on 'Detection times and number densities of rare mobile organisms: application to Loch Ness'.

If there are any, could there be many?

IN simple collision physics, when one moving object may collide with a number of stationary and dissimilar targets, the mean free time for collision is $t = (n\sigma v)^{-1}$, where n is the number density of stationary targets, σ is the mean collision cross-section and v is the relative velocity (assumed constant). If targets are also moving, a factor of order unity multiplies the denominator of this equation; for example, if all objects have Maxwell-Boltzmann velocity distributions, the multiplier is $2^{1/2}$.

This same equation, slightly modified, can be used to deduce the number density in three dimensions of a distributed rare mobile organism from the mean free time between sightings of this organism by a stationary or moving observer. In this case the collision cross-section $\sigma = \pi r^2$ where r is the target visibility range—the linear distance over which the target is within the resolving power of the observer; or the distance to optical depth unity in the enveloping medium; or the distance to the horizon, whichever is least. When t is measured by a stationary observer, the mean distance between organisms will then be

$$S = 2(3t r^2 v/4)^{1/3} \quad (1)$$

If the total volume in which the organisms are contained is V , the total population of organisms in this volume is

$$N = V/(\pi r^2 vt) \quad (2)$$

These relations assume that the organisms being observed are neither attracted to nor repelled by the observer, and that the observer has chosen a typical locale in the organism's habitat—for example, not in the vicinity of concentrations of predators or prey. Under these circumstances equations (1) and (2) provide expectation values for the mean separations and total numbers of organisms. In the common case that the geometry is two- rather than three-dimensional (as, for example, for land animals and to a significant extent even for birds), n is replaced by N , the column density of organisms, σ is replaced by r , $S = 2(rvt/\pi)^{1/2}$ and $N = A/rvt$, where A is the area of the total habitat.

As a practical application, consider the interesting and controversial set of observations suggesting the presence of large organisms in Loch Ness¹. Sonar and underwater stroboscopic photography in 1975 imply $t \approx 10^4$ to 10^5 s

for some unidentified large animal of characteristic dimensions 10 m. We adopt $t \approx 3 \times 10^4$ s, but bear in mind the impression of the observers that in 1975 the organisms may have been attracted by the observational equipment and therefore that the appropriate t is significantly longer. Because of the turbidity of the loch, $r \approx 10$ m; and a rough estimate of the swimming velocity of the unknown animals is $v \approx 3 \text{ m s}^{-1}$.

Equation (1) then immediately gives $S \approx 0.4$ km, a very large mean separation distance. The total volume of Loch Ness is approximately 10^{16} cm^3 , whereupon, from equation (2), $N \approx 300$. Because of the cube root in equation (1), our estimate of S is reasonably independent of the uncertainty in t . But our uncertainty in estimating the total population is proportional to the uncertainty in our estimate of t . If the targets were indeed attracted to the observing apparatus, then N is less, and a conservative estimate places N between several tens and several hundreds. Curiously, this is just the estimate derived independently from biomass calculations, assuming that the diet of the unknown organisms is exclusively migratory salmon² or exclusively non-migratory prey³. While the agreement of these two quite different sets of calculations—from the waiting time for observation in 1975 and from the biomass of the loch—should not be overstressed, the agreement does tend to support the contention that there is a real population $\approx 10^{2 \pm 1}$ of large organisms

inhabiting Loch Ness.

The negative photographic results for 1976 have been attributed⁴ to the sharply diminished fish population and significant increase in temperature in the loch, connected with the 1976 drought. If the 1976 results were not anomalous, we might deduce $t \geq 10^7$ s, and $N \leq 1$. However, sonar encounters at larger r were recorded in 1976.

The nature of these organisms seems still more uncertain than their existence, but it appears more likely that they are a minor variant of a fairly abundant contemporary taxon than, for example, the only surviving group of aquatic Mesozoic reptiles. The large calculated separation distances in a medium as turbid as Loch Ness suggests that the organisms might be equipped with echo locator organ systems and may communicate at audio frequencies. Hydrophones should be an important adjunct to any continuing study.

Similar calculations of organism spacing and loading density could be made on other planets, were macro-organisms to be discovered there—as, for example, on Mars with the Viking lander imaging system. □

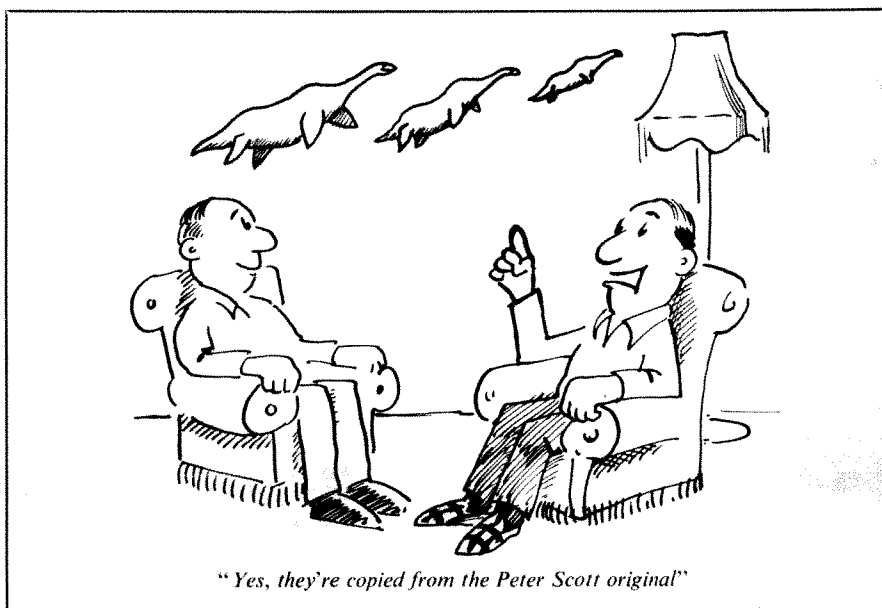
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"Yes, they're copied from the Peter Scott original"

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USA

New career for Glomar Explorer?

The National Science Foundation is looking into the possibility of converting the Central Intelligence Agency's Glomar Explorer into a deep sea drilling vessel. Colin Norman reports from Washington.

WHEN it became known in March last year that the Central Intelligence Agency (CIA) had used a vessel called the Glomar Explorer to scoop bits of a sunken Soviet submarine from the bottom of the Pacific, a number of scientists in the United States expressed concern that the affair could jeopardise flourishing Soviet-American cooperation in deep sea research. A particular worry was that the Soviet Union might sever its ties with the deep sea drilling project (DSDP), a highly successful ocean drilling programme which the National Science Foundation was then endeavouring to turn into an international research enterprise. Ironically, however, the DSDP may turn out to be the chief beneficiary of the CIA's salvage operations.

Last week, the National Science Foundation (NSF) announced that it is studying the possibility of converting the Glomar Explorer into a drilling vessel for possible use in the 1980s. NSF officials, and their clients in many earth science laboratories, are hoping that the Glomar Explorer will provide a cut-price way of carrying out drilling projects which are beyond the capabilities of the present DSDP vessel, the Glomar Challenger. Though it is far from certain that the NSF will be able to get the funds to turn the vessel into a research ship, it is the only use for the ship which is now under serious investigation.

The Glomar Explorer was constructed for the CIA by Global Marine Inc (the same company which built and operates the Glomar Challenger, hence some of the worry about Soviet scientific reaction), for a sum reported to be about \$300 million. Though publicly billed as a special seabed mining ship built by Howard Hughes, the reclusive billionaire, it was designed for the specific purpose of salvaging a Soviet submarine which sank in about 1,700 feet of water 750 miles north-west of Hawaii in 1968.

The ship recovered bits of the submarine in June 1974—how much is still a matter of some dispute—and when reports of the operation began leaking to the press nine months later, the CIA suddenly found itself with an almost new vessel for which it could

find no further use.

In the meantime, earth scientists have begun to think about what should follow the present phase of the DSDP. The choice boils down to either dispensing with deep sea drilling entirely or mounting a programme of drilling in presently inaccessible areas such as potential oil-bearing regions, the ocean trenches and under the Arctic sea, a programme which would require a new vessel with greater capabilities than the Glomar Challenger. It would require considerable lifting capacity to handle the long drill strings needed for drilling in ocean trenches, and a thick hull to penetrate the Arctic ice. Moreover, since drilling in the ocean margins entails the risk of striking gas or oil deposits, the vessel would have to be able to handle complex and heavy apparatus to prevent possible blowouts. Construction of such a vessel would probably cost between \$60 and \$70 million, a price tag which probably puts it out of the reach of NSF's strained budget.

But Peter Wilkniss, the NSF project director for the DSDP, noted in an interview last week that "when you look at the requirements, it becomes obvious that the Glomar Explorer is almost ideally suited." It is 618 feet long, compared with 400 feet for the Glomar Challenger, and it is capable of lifting about 2,000 tons—more than 10 times the capacity of the Challenger. Moreover, its more powerful engines and greater stability would probably allow it to work in rougher seas.

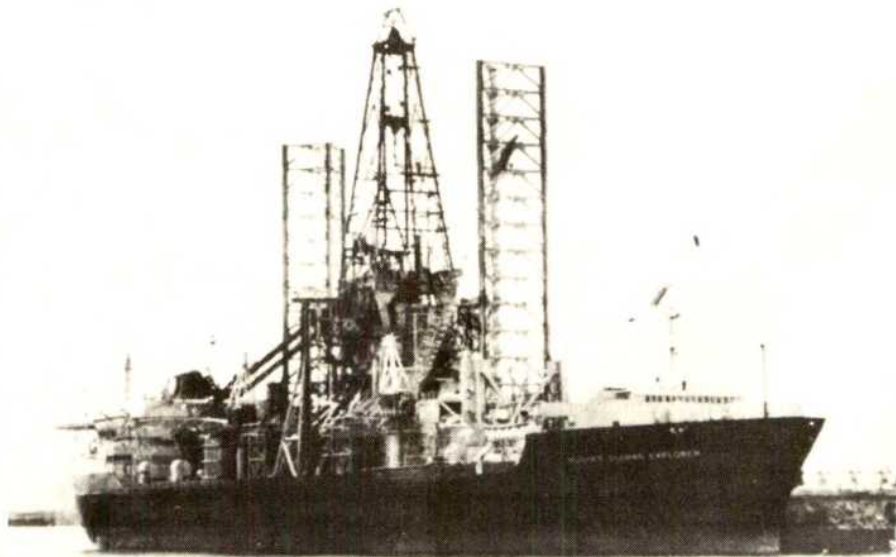
It is by no means certain, however, that the vessel could be converted

easily and cheaply from a salvage ship into a deep sea drilling vessel, and thus NSF has authorised Global Marine to conduct a preliminary study, under a \$75,000 contract, of the feasibility of converting and operating the Glomar Explorer. The results of that study should be available early next year. In the meantime, the Scripps Institution of Oceanography, which manages the DSDP under a contract from NSF, is looking at the scientific requirements of an expanded drilling programme.

So far, NSF has not given much thought to arrangements for operating the ship if it does decide to acquire and convert it. But, in view of the potentially high costs of operating the vessel—Wilkniss estimates about \$30,000–40,000 per day, twice that of the Glomar Challenger—there would be obvious advantages in seeking international participation.

In that case, the Glomar Explorer's past associations could pose a problem. Though the Soviet Union did not pull out of the DSDP after the submarine salvage attempt was made known, the operation is said to be a sensitive matter and some ocean researchers privately wonder whether the Soviet Union would participate in a venture for which the Glomar Explorer was a central feature.

It should also be noted that if NSF does decide to follow the present DSDP with a programme of drilling in the ocean trenches and on the continental margins, much of the work would be carried out within the 200-mile economic zones likely to be established as part of a Law of the Sea agreement. That would subject the research to a possible veto by coastal states in whose waters the Glomar Explorer may want to work. □



Glomar Explorer

USA

● The Joint Committee on Atomic Energy, the Congressional body which has reigned supreme over nuclear policy in the United States for nearly three decades, last week suffered a severe, possible fatal public battering. Already weakened by the loss of several key members through retirements and election defeats, and facing concerted attack from senators and congressmen seeking its abolition, the joint committee last week became the target of some well-aimed barbs hurled by Common Cause, an influential lobbying group.

In a lengthy review of the joint committee's activities, Common Cause says it has uncovered numerous examples of "procedural abuse", pro-nuclear bias, and failure to examine important issues. The harsh indictment may turn out to be an important factor in persuading Congress that the time is ripe to carve up the joint committee's responsibilities and divide them among other congressional committees, a move which is already being planned and which would have important implications for nuclear policy.

The joint committee derives much of its immense power from its unique status as the only Congressional committee which handles legislation in both the House and the Senate. Every bill related to nuclear power, no matter where it originates, must be referred to the joint committee, a fact which gives its 18 members a monopoly over nuclear legislation.

Formed shortly after the second world war to oversee the United States's nuclear weapons programme, the joint committee rapidly became one of the most powerful committees in Congress. For more than two decades its authority went largely unchallenged and legislation it approved was virtually guaranteed easy passage through the House and Senate. But, as nuclear power has turned into a controversial issue, the joint committee has attracted increasing fire from nuclear critics. It has been persistently accused of pro-nuclear bias, and in the past couple of years a few other Congressional committees have even begun to invade its turf by holding hearings on nuclear matters. (The joint committee is still the only committee with authority to consider nuclear legislation, however.)

Criticisms of the committee have recently given way to more strident calls for its complete abolition, and the Common Cause blast is no exception. The organisation's report pre-

sents evidence that the joint committee has given short shrift to the concerns of nuclear critics, failed to explore important nuclear issues which raise doubts about nuclear power, refused to take the lead in examining major policy questions—such as nuclear proliferation—and often used its unique Congressional position to rush legislation through both the House and Senate before



relevant studies have been completed.

Common Cause also notes that the committee has persistently voted for increases in nuclear spending, adding more than \$850 million to the budget of the Energy Research and Development Administration (ERDA) in the past three years alone. Those actions, Common Cause claims, can be explained in part by the fact that the committee is "dominated by representatives who have a vested interest in encouraging increased nuclear spending". In the last session of Congress, for example, 12 of the committee's members represented six states which together carried off more than half the funds distributed by ERDA.

The chief recommendation from Common Cause is that the joint committee should be scrapped and its responsibilities transferred to House and Senate committees which have broad authority over all energy research and development matters. The report is likely to give considerable impetus to moves already afoot which would accomplish precisely that objective.

Next month, when Democrats in the House of Representatives meet to consider their political agenda, Representative Jonathan Bingham will propose that the joint committee be scrapped and its responsibilities be consigned to other committees, probably the Science and Technology Committee or the Interior Committee. And a committee reorganisation plan

proposed recently for the Senate would transfer the joint committee's responsibilities for nuclear research and development to an expanded Interior committee, and its authority over nuclear regulation to a new Public Works and Environment committee. The reorganisation plan is expected to be debated by the Senate in January, as one of its first items of business.

● If the Joint Committee on Atomic Energy is scrapped by the new Congress, the nuclear industry in the United States would lose one of its most important political allies just as a number of matters deemed crucial to the industry's long-term interests are coming to a head. Among those issues is whether the United States should press ahead with the liquid metal fast breeder reactor programme, at present the highest priority and most lavishly funded energy research and development effort supported by the federal government.

In that regard, a report published last week by the General Accounting Office (GAO), an investigative agency of the Congress, is unlikely to help the LMFBR's political acceptability. The report estimates that it would cost some \$150,000 million to build the 128 breeder reactors which ERDA is now planning, compared with \$95 million to build coal plants of an equivalent capacity and \$128 million for light water reactors. That level of capital expenditure would be difficult for the electricity industry to raise without federal assistance.

● There has recently been a spirited debate in many countries about how to dispose of commercial nuclear wastes and keep them isolated from the environment for thousands of years. The disposal of nuclear wastes from military programmes in the United States is, however, an even bigger problem. Last week, the Energy Research and Development Administration announced that it hopes to start drilling next year in some 13 states to find suitable sites for commercial repositories. Though no sites have yet been short-listed, ERDA officials said that they hope to have the first repository in operation by 1985, and some six in operation in the 1990s. The total cost of the programme, which will handle some 330,000 cubic feet of material by the year 2000, is reckoned to be \$2,000 million. Under questioning, however, ERDA officials said that the total amount of waste generated by military programmes by 2000 will be about 11 million cubic feet, and the cost of getting rid of it could be as much as \$20,000 million.

Colin Norman

FRANCE

La Soufrière's first victim

Fierce disagreement characterised the debate amongst French scientists about La Soufrière, the volcano in Guadeloupe in the French Antilles. A report from La Recherche:

WHEN La Soufrière started to grumble in July 1975 the memory of the 30,000 victims who perished in the cloud of burning ash thrown out by Pelée Mountain in Martinique in 1902 made the question of evacuation particularly important. Some 72,000 people were living at the foot of La Soufrière, which is one of a group of volcanoes in the Caribbean island arc.

Its activity has been characterised by its andesitic nature, the relative viscosity of its magma, the long periods of calm between eruptions and particularly by the explosive nature that its eruptions can have. Although known eruptions (1797, 1836 and 1956) had produced only a slight rain of volcanic dust and released some steam, the authorities eventually did evacuate the local population as a preventative measure. But after a period of intense activity in July and August, the volcano seemed to quieten down again. Things should then have returned to normal; in fact the trouble had only just begun.

On October 27, the board of the Teaching and Research Unit (UER) of the Institute of Physics of the Globe (IPG) met to discuss with the Director of the Institute, M Allegre, the case of Haroun Tazieff, the famous French volcanologist. Allegre informed the board that he had decided to dismiss Tazieff (63) from his post as director of vulcanology at the IPG. Tazieff, he explained, had refused to guarantee that he would fulfil the commitments of the post, after being asked to do so several times and in particular at the beginning and end of August. That followed criticisms of Tazieff for going off to hunt for three Britons lost in the Andean Cordillera when 72,000 French people could have been in danger.

Although no vote was taken at the meeting those present unanimously approved the measure. As the IPG does not come under the administrative jurisdiction of the National Centre for Scientific Research (CNRS), Tazieff continued as a research director at CNRS; his team was anyway in the process of moving to Gif sur Yvette outside Paris to be on CNRS premises. The break with the IPG was complete, though, because Tazieff had no desire to remain any longer on its staff.

The episode marked the culmination of an unfortunate conflict between French scientists which had for several months taken precedence over the real problems confronting the people of Guadeloupe, already faced with considerable political, social and economic difficulties. The UER has given its official reasons for the dismissal of Tazieff, but some believe it may be the result of a quarrel which has been exacerbated by a series of petty disagreements over the differing interpretations of the phenomena observed on the volcano itself.

Tazieff's own somewhat strong words, which the press willingly echoed, started the quarrel and made it all the more bitter as time went on. In *Le Monde* (September 5) he declared that he "could expose the faulty reasoning which has led certain scientists not competent in vulcanology to put forward mistaken prognostications, which the volcano itself has proved wrong in the past six weeks, but I will only do so in a scientific journal." Five days later, there were even stronger words in the weekly *L'Aurore*: "Those who blocked my way are incompetent," he declared. "They are all perfectly competent in their own fields, but they should not try to interfere in vulcanology. If you have trouble with your liver you do not consult an ophthalmologist."

The confrontation deepened on the scientific plane. From July 1975 La Soufrière manifested an increasing number of the phenomena which have now died down again: a seismic shock in March, the appearance of new fractures, underground phreatic eruptions (in July), exceptional seismicity, stronger and more frequent eruptions (in August). Over this period, the number of seismic shocks registered rose from 30 in July 1975 to 209 in November, 607 in March this year, 1,220 in July and, in August, almost 6,000 tremors.

The scientific community agreed on these facts, but there was no unity about the diagnosis. On the one side, Professor Robert Brousse declared that he could no longer rule out the possibility of an explosive phase which could come very suddenly. John Tomblin of the University of Trinidad supported this viewpoint. On the other hand, Tazieff was more reassuring, saying that there was no need to fear a rapid development, and that the only risks for September were from the underground eruptions which endangered only the immediate vicinity



La Soufrière

of the volcano. "Any major eruption," he added, "will be preceded by warning signals for about a week."

On August 15, after a strong tremor of magnitude 4.6 shook Guadeloupe, the political authorities organised a massive evacuation of the population. Even though Allegre wished to hold a middle view between the two extremes, in effect he confirmed the alarmist views of Brousse when he sacked Tazieff, describing his statements as unscientific.

A committee of international experts met in Paris in the middle of last month, brought together by the CNRS to give its opinion on the surveillance programme for the volcano, its organisation, and the results obtained. During these meetings, members have been listening to the many French and international scientists who have been involved with La Soufrière.

It appears from the discussions that there is no danger in the short term of any sudden explosive phenomenon, and that all changes in the behaviour of the volcano should be easily registered by the modern instruments available. It is essential, the committee has noted, that the equipment at La Soufrière should be improved and reinforced. This means that the number of seismograph stations in the existing array should be increased from seven to about twenty, and that they should be set out around the circumference of the volcano. It was also agreed that magnetometers, tiltmeters and inclinometers should be installed.

The committee also advised that to utilise the installations best, a volcanologist and a petrologist should, as an experiment, be employed permanently in the area. They would look after the equipment, make daily observations and do regular analyses of the material thrown up by the volcano. This would avoid, the committee hoped, the danger of over-reacting when there were insufficient data. □

USSR

Bottling up dissent

Vera Rich reports on recent developments concerning dissidents in the USSR and Eastern Europe.

LEONID PLYUSHCH, a former prisoner of conscience who was interned in the Dnepropetrovsk psychiatric penal institution, visited London recently. His presence formed a rallying point for all those concerned in the cause of human rights and freedom of thought in the USSR and the Comecon bloc.

Plyushch, a cybernetician who was arrested in 1972 for "anti-Soviet agitation and propaganda", was tried in his absence and interned as suffering from "creeping schizophrenia". His release was effected in 1975 following a massive campaign both from the mathematical and psychiatric communities abroad. Speaking in his native Ukrainian, a medium of expression which he explained was itself a symptom of "dissidence" to Soviet forensic psychiatrists, Plyushch declared that he did not wish to recall the details of his incarceration—the massive doses of neuroleptic drugs, the ambience of the genuinely sick and the "doctors" whose

white coats only partially covered their police uniforms.

In an interview with *Nature* he indicated he was concerned rather to campaign for those still in captivity, either his fellow internees in mental institutions or those who, like Vladimir Bukovskii or Semeon Gluzman, are now serving prison sentences for bringing the Soviet abuse of psychiatry to the attention of the world scientific community. Some 1,000 detainees, he estimated, are incarcerated in penal or ordinary mental institutions simply because their views on politics and human rights do not coincide with the official norms. Asked whether he had any regrets in his own case, Plyushch replied that, speaking with hindsight, he could now see that it would have been advisable to proceed more "canily", to be less open and naive, and to make sure that the authorities had less opportunity to gather a dossier against him.

Having acted as a liaison officer between the movement for self-determination in his native Ukraine and the Sakharov human rights committee in Moscow, Plyushch gave some interest-

ing new sidelights on the dissident movement. He stated that the campaign for human rights and civil liberties was primarily a movement of the scientific community, and had as yet little grass-roots following, thus differing sharply from the movement for religious liberty and the "national" movements in the Union (that is, non-Russian) Republics, which included all sections of the community. Only in certain specific areas, notably in West Ukraine (incorporated into the Soviet Union in 1939) is there a close link between the demand of the workers and peasants for better living standards and the campaign of the intellectuals for greater democratisation "on the Czech model".

As a cyberneticist himself, Plyushch stressed that the scientists in the dissident movement must put forward a "new kind of planning" if their ideas are to command the kind of mass support that he sees as the only sure foundation of democratisation.

The kind of union of workers and intellectuals which Plyushch advocates is an important feature of the new protest movement in Poland. Following the protests of workers in the Radom and Ursus plants against rising food prices, a defence committee was

A letter from the USSR

Nature has received this letter, addressed "to all scientists of the world":

Dear colleagues,

By this letter we want to call your attention to the tragic fate of biologist Sergei Kovalev. We want scientists to have a clear realisation of the conditions under which those who venture to protest against arbitrary rule are compelled to live in the Soviet Union. By the nature of their profession scientists strive for adequate cognition of the world. That is why we hope that it will be scientists who gain the best insight into certain features of the Soviet regime. This understanding could be of great importance.

Sergei Kovalev is a gifted scientist, specialist in the field of heart and cell physiology. In December 1974 he was arrested, and in December 1975, after a year of imprisonment, sentenced to seven years of prison camps of severe regime to be followed by three years in exile.

Kovalev's alleged "crime" was that in accordance with his convictions he could not and did not obey an unwritten Soviet law: to keep silent about all acts of arbitrariness and injustice done by Soviet authorities. Kovalev

was one of the first to join the Human Rights Movement. In May 1969 he entered "the Initiative Group" for protection of human rights. At a moment when "The Chronicle of Current Events" (a typewritten journal of political repression in the USSR) was savagely persecuted, Kovalev together with T. Velikanova and T. Khodorovich courageously announced that they would do their best to disseminate this journal since it gives true information about the violations of human rights in the USSR. All his activity in protection of human rights was imbued with noble spirit and humanism.

Sergei Kovalev committed no crime. In any open society he would be one of its most respected members. The Soviet authorities declared Kovalev a dangerous criminal. His imprisonment is not a mere limitation of freedom. In the camps they are trying to "rectify" Kovalev's convictions by isolation, hunger and humiliation. A renowned scientist is forced to do exhausting monotonous physical work, he is limited in correspondence (two letters a month), in visits of close relatives (one visit every four months at most, in practice still less). Malnutri-

tion also is one of the "rectifying" measures; accordingly, during three and a half initial years Kovalev will not be allowed to receive parcels.

But all this does not seem enough. Kovalev is constantly persecuted by the camp administration. He is not given qualified medical treatment and has no possibility of curing a painful chronic disease which makes his prison life unbearable, hard enough as it already is.

We call scientists of the whole world to make use of every opportunity to draw public attention to the tragic fate of Sergei Kovalev.

We call scientists to appeal to Soviet legislative, governmental and party bodies on behalf of Sergei Kovalev.

We call biologists to withhold scientific contacts with the Soviet Union until Sergei Kovalev is released.

ANDREI SAKHAROV, VALENTIN TURCHIN, YURI ORLOV, YURI MNYUKH, NINEL PANFILOVA, YURI GOL'FAND, VENYAMIN LEVICH, GALINA SALOVA, YURI GASTEY, ALEKSANDR LAVUT, TATYANA VELIKANOVA, ALEKSANDR LERNER, TATYANA KHODOROVICH, IGOR MEL'CHUK, ALEKSANDR KORCHAK, VLADIMIR ALBREKHT, NAUM MEIMAN, EFREM YANKELEVICH, ELENA BONNER, YURI SHIKHANOVICH, MARK AZBEL'.

set up, including writers, lawyers, historians and scientists. Among those already subjected to reprisals, biochemist Piotr Naumski has already suffered a short term of arrest, while Mirosław Chojewski, a chemist, has been dismissed from his post at the Institute of Nuclear Research in Swierk.

A letter from the Defence Committee to Academician Andrei Sakharov, appealing to his "moral authority and fellow-feeling", evoked a declaration from him that although he did not know what concerted actions could, at the present time, be aligned with the actions of the Polish defence committee, he was nevertheless seeking ways in which the Soviet human rights movement could "effectively cooperate" with similar movements throughout Eastern Europe.

There seems to be enough dissident activity to suggest that the time is ripe for such a concerted effort, if only one of moral support. In the Soviet Union a press campaign has been launched against Aleksandr Voronel, the founder of the illegal "Sunday seminar" for Jewish refusniks, denouncing him as a traitor. The seminar, which survived Voronel's departure for Israel in December 1974, is now approaching its fifth anniversary, and is planning a

special Jubilee session (Sakharov is to be on the jubilee Committee). Nevertheless pressure on the Seminar continues; recently several of its leading members, including physicist Mark Azbel, the organiser, were held by the police for several days.

In East Germany, the chemist Robert Havemann is subject to house arrest, for having protested against the exiling of Wolf Biermann, the dissident poet and ballad-singer. Havemann's dissident "career" dates back to 1964, when he was dismissed (on account of his "revisionist" views) from his post as Professor of Physical Chemistry at the Humboldt University, Berlin.

From Bulgaria it is understood that an attempt to disseminate some controversial material in *samizdat* form led to the removal of their author (a physicist) to a mental hospital. Although he was subsequently discharged, he was not reinstated in his university post but was instead retired on a disability pension, thereby effectively removing his ability to speak from a position of authority.

This appears to mark a new development. The dissemination of Soviet technology and ideas among the countries of the Socialist block is a basic concept of Comecon. So far, however, the incarceration of dissidents

in mental institutions has been primarily a Soviet phenomenon, although there have been hints of isolated cases in Czechoslovakia and the German Democratic Republic.

● Lord Todd had some words to say last week on the Royal Society's role regarding scientists and human rights. Delivering the Society's Anniversary Address, he said it was hard to see in what way the Society could occupy a special position. He pointed to the Society's adherence to the Declaration issued by the International Council of Scientific Unions and by its cooperation with that body in efforts to uphold it in all its member countries. In appropriate cases, he said,

the Society has drawn and will continue to draw the attention of the Soviet Academy of Sciences or the corresponding body in any other country concerned, as well as our own Government, to the facts and to the need for action with, I believe, good effect. It is my firm belief that the Society as such can achieve much more in this way than it can by subscribing to or issuing public declarations. For it must be recognised that a scientist, as such, is in the same position as any other citizen of his country, subject to the same laws and having the same obligations to the society in which he lives. His profession does not entitle him to special privileges which are denied to his fellow citizens; nor should it deny to him those privileges which are the right of every man. □

WEST GERMANY

Reactor reactions

A Correspondent reports on recent developments on the nuclear power front in the German Federal Republic.

THE future of nuclear power in West Germany depends critically on imminent decisions over the construction of nuclear reprocessing and storage facilities in Lower Saxony. The rumble of anti-nuclear feeling, which recently erupted in demonstrations at the Brokdorf power station site, led the Interior Minister, Werner Maihofer, to announce earlier this year that no new permits for the construction of atomic power stations would be issued until firm plans existed for dealing with spent fuel. The salt mines of Lower Saxony are considered the only suitable site for long-term waste storage. The Prime Minister of Lower Saxony was begged four weeks ago by Bonn Cabinet ministers to allow the plant to be built and seems to have been persuaded: the proposed site of the plant is to be announced soon. But determined local opposition will ensure that its path will not be smooth.

The 20% of Germans against nuclear energy probably entertain strongest fears about reprocessing and storage of nuclear fuels. But the most violent

protests have been not in Lower Saxony but at the site 50 km north-west of Hamburg where Kraftwerk Union is beginning to build West Germany's latest nuclear power station. Brokdorf, on the Elbe, has seen two ugly confrontations between demonstrators and police in the past few weeks, with many injured. The protests are not against any particularly unsavoury feature of Brokdorf but rather against nuclear expansion in general and the way industry is steadily transforming the lower Elbe region into another Ruhr.

The protests could equally well have been focused on Biblis, another Kraftwerk Union product and the world's biggest nuclear plant, which was forced to shut down for three months during the summer after screws had been found dislodged from a cooling pump. An even more controversial aspect of the plant is the intention of the owners, Rheinisch-Westfälische Elektrizitätswerke (RWE), to increase storage capacity for spent fuel pending construction of a reprocessing plant in Germany—RWE apparently find French and British reprocessing too expensive and prefer to sit on their fuel for an extra five or six years until the first German reprocessing plant is in action. Another worry for safety-con-

scious Germans is the prospect of the reactor which the chemical company BASF is to build in an urban area at Ludwigshafen. And a third reactor—that planned for Wyl on the Rhine—is as yet unstarted because of public protest. Even discounting the political element in the demonstrations, it is clear that the new anti-nuclear feeling is not to be taken lightly.

This is recognised by the Free Democratic Party, the junior party in West Germany's coalition government, who have voted at their party conference to put strict limits on new nuclear construction. Though the vote has no binding effect for the government it is thoroughly awkward for them and in particular for the Economics Minister, FDP member Hans Friedrichs, a strong nuclear supporter.

Altogether embarrassing timing for Siemens's announcement of its intention to take on AEG-Telefunken's 50% of Kraftwerk Union, the leading West German power station constructor, thus becoming sole owner. But Siemens is probably the happier of the two concerns. It is confident about the future of nuclear energy and has the resources to see the voracious and currently unprofitable Kraftwerk Union through the next few lean years. AEG-Telefunken, though glad to be rid of what it sees as an albatross, is not getting a particularly good deal. □

IN BRIEF

CERN subscription

At the time of going to press, it seems that the crisis over British international subscriptions, particularly to CERN, has been resolved. The problem arose because the government has been operating a strict cash limits policy, whilst subscriptions to international bodies have grown with the decline in the pound. In the Winter Supplementary Estimates, however, it has been possible to raise the Science cash limits by £2.5 million whilst dropping the University limits by the same amount. This step will not affect any university grants already announced — it

comes from an operational reserve now regarded as more than adequate. The remaining £3.5 million needed to fulfil the subscriptions comes from a variety of sources, the main being a CERN refund of £0.6 million on previous programmes and a re-examination of allowable accounting procedures for research councils in dealing with commissioned research.

Swedes propose nuclear law

New Swedish power stations will not be able to begin operating until they can

demonstrate that reprocessing and disposal of spent fuel can be carried out "in a completely safe manner" if a proposal made by the government last week becomes law in March as expected.

All companies building nuclear power stations would have to specify in detail in what form the spent fuel would be deposited and how the radioactive waste would be transported. The legislation would not affect the six Swedish reactors already in action or being loaded in preparation for action, but would apply to the four at present under construction.

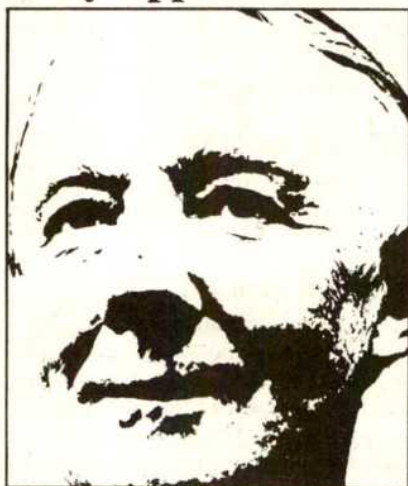
SOME things are done very differently in the United States and in Britain. We are told that Mr Jimmy Carter, the President-elect, will have to appoint some 3,000 people in various offices. Many of those chosen may be rewarded for political services, others may be apolitical and some may even, in the past, have been his opponents. Though Mr Carter will not himself be able to choose each individual personally, he clearly bears a direct responsibility for the whole operation.

In Britain, particularly in the scientific field, changes of government have no immediate effect on the majority of appointments, and there is no wholesale turnover of office holders following a general election. Different governments have made many changes in the pattern of scientific management, generally with unfortunate results, but these have usually been implemented by a process of "general post" with the same faces reappearing with different titles and slightly different responsibilities.

Britain differs most from America in that the way in which many appointments are filled is shrouded in mystery. The full-time senior scientific posts, within government ministries, are mostly filled by the normal civil service machinery, though in many cases the methods by which the office holders are selected are as closely guarded as the register and membership of Cabinet committees. However, the results of this method are generally satisfactory, and sometimes brilliantly successful.

What is even less well understood, however, is the way in which the chairmen and members of influential bodies like our research councils or of the Nature Conservancy Council (whose ancestor was an independent research council until 1965) are selected. Although there are some very square pegs which have been thrust into round holes, in most cases the choice of members has been un-

exceptionable. Some have been active in science and have done distinguished research; a few are still active in their own fields. What is more doubtful is whether they are always the right men (I use the word deliberately, for few are women) to

By appointment**KENNETH MELLANBY**

carry out the duties now apparently expected of them.

It is generally believed that the idea of the independent research council, financed by government, was a good one, and did much to foster high-grade research in Britain. Originally the councils included laymen, some even being politically active members of both Houses of Parliament. The councils' functions were exercised by comparatively infrequent meetings to determine, or more often to approve, major questions of policy, and they interfered little with the general organisation of research and never with the actual work of the scientists they employed. From time to time there were criticisms, such as that even their most senior scientists were excluded from

membership of their own research councils.

I once heard this justified by a member, who said that it would clearly be improper for an employee to be a member of his own governing body. The speaker was a professor much engaged in university affairs; he was temporarily disconcerted when I suggested that his membership of his university's Council and Senate was, by analogy, equally improper. However, most of the active scientists probably preferred the freedom of their laboratories, and seldom hankered after public office.

Today members of these councils seem to be expected to take a much more active part in their work. Many of them are now paid a modest salary which, being honourable men, they feel they must earn. So meetings of the councils themselves, of their committees, working parties and preparatory groups take place interminably. The employed staff, including scientists who should be doing full-time research, spend more and more time "servicing" these meetings (though they are generally excluded from the more important sessions). My general impression is that all this extra work is almost entirely counter-productive, resulting in less and worse rather than more and better research. This is surely what might have been expected. Where research is concerned, no committee has ever had an original idea. The more such committees meet, the more they are likely to obstruct research.

Research councils are now complaining about their shortage of funds. If they ceased to pay their own members and if they met less often this would set free a modest sum to pay a few more bench workers. But more important, the reduction in the time wasted on and by the committees would do much to ensure that a greater proportion of the funds could be spent more fruitfully.

correspondence

Lavoisier's originality

SIR,—Antoine Laurent Lavoisier is generally credited with the independent discovery of oxygen and its role in combustion and respiration. But the existence of his personal copy of the complete works of John Mayow¹ suggests that his ideas were not as original as he claimed. Lavoisier's early detractors seem to have been justified even when they accused him of a lack of originality.

Mayow recognised the existence of oxygen, which he called *Spiritus nitro aereo*, in 1674 when he was experimenting in physiology at Oxford. He described the decrease in the "portion of air that made respiration possible and was essential to life as well as to the combustion of candles". He also described quite accurately the changes occurring in red blood cell pigment exposed to either "fresh" or "corrupted" air. He considered that the portion of air that made it "respirable" was fixed to this coloured compound in the blood cells and acted as an indicator of whether the blood was "respirable" (red, arterial blood) or "corrupted" (dark purplish, venous blood).

There are striking similarities between the writings of Lavoisier² and Mayow on this subject, although the former insisted that he did not know the work of the latter³. Some eminent eighteenth and nineteenth century scholars, however, including Cuvier⁴, maintained that he did, and Hoefer⁵ considered that Mayow was the "real father of modern chemistry and the inventor of the theory of respiration".

Lavoisier seems indeed to have known about Mayow's work because a letter³ to him from Mr Koenig of Strasbourg, dated 1767, contained an invoice for 118 books, among them a copy of Mayow's *De Sal Nitro*. As Mayow's tract *De Sal Nitro* was only published in the *Opera Omnia Medico-Physica* one must conclude that this book was the *Opera Omnia*. The book I found recently in the library of the Académie des Sciences de Paris, with the inscription 'De la Bibliothèque de M Lavoisier, Registre de Poudres et de Salpêtres de France, Inspector Générales du Roy', was one of the 1681 edition of *Opera Omnia*, published in The Hague. This book was in Lavoisier's personal library and then passed on to J. B. Dumas, who died in 1884. Dumas greatly admired Lavoisier and arranged for the publication of his works⁶. In

1937 the book was given to the Académie des Sciences.

Lavoisier, therefore, must have been familiar with Mayow's work when in 1777 he presented his memoirs on oxygen and respiration² to the Académie des Sciences, although in 1776 a letter from M Magalhaes⁷ to Lavoisier suggests that Lavoisier was only then looking for Mayow's works. His subsequent protestation of ignorance³ seems to have represented an eighteenth century version of scientific cheating.

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¹ Mayow, J., *Opera Omnia Medico-physica Tractatibus Quinque Comprehensa* (Leers, the Hague, 1681). See Partington, J. R., *A History of Chemistry*, 2, 582 (Macmillan, London, 1961).

² Lavoisier, A. L., *Memoires sur la Respiration et la Transpiration des Animaux*, 185 (Académie Royale des Sciences, Paris, 1777).

³ *Oeuvres de Lavoisier*, 7, Letter No. 59 (edit. by Fric, R.) (Editions Albin Michel, Paris, 1955).

⁴ Cuvier, G. L. C., *Histoire des Sciences Naturelles* (Masson, Paris, 1841–1845).

⁵ Hoefer, J. C. F., *Histoire de la Chimie*, 2, Tome II (Hachette, Paris, 1842).

⁶ *Oeuvres de Lavoisier*, 1–6 (edit. by Grimaux, E.) (Imprimerie Impériale, Paris, 1864).

⁷ *Oeuvres de Lavoisier*, 7, Letter No. 320 (edit. by Fric, R., Daumas, M., and McKie, D.) (Editions Albin Michel, Paris, 1964).

Amazon rain forests

SIR,—The Amazonian rain forests are indeed "one of the world's great photosynthetic factories", as Thomas Jukes has recently stated (November 11, page 108), but he is wrong to add that they "maintain atmospheric oxygen". The total area of the Amazon basin rain forests is about 3×10^6 km² and the above-ground biomass is on average about 25,000 tonnes km⁻², but this huge mass of vegetation is not a net source of oxygen. Overall it is in equilibrium just as is any substantial area of climax vegetation.

Patches in the building and mature phases of the forest growth cycle do show net increase of fixed carbon and an accompanying release of oxygen, but this is balanced by decomposition of dying vegetation, in which oxygen is taken up, in patches of overmature forest and the following gap phase of the growth cycle. Man-made or natural catastrophes in which large areas are cleared and the vegetation destroyed by burning or natural processes of decomposition do locally and temporarily lead to fixation of oxygen, but these are followed by a vigorous regrowth of vegetation with a rapid build-up of biomass during which oxygen is released.

So the luxuriant regrowth which soon refills a jungle clearing is actually for a time a source of oxygen. What are altered by large scale clearance are the structure and species-composition of the forest, and unnaturally extensive clearance by modern man could progressively deplete the forest of its diversity and lead to reduction in numbers or even extinction of some plants or animals. There is also the possibility of altering reflectivity of the surface, hence the heat balance, hence, conceivably, local climate (Stewart P., *Commonw. For. Rev.*, 55, 155–157; 1976). These are the dangers to be countered as "development" of the rain forest takes place.

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Seveso: putting it right

SIR,—With Alastair Hay's article on Seveso (October 14, page 538) is an item on alternatives to 2,4,5-T in which it is stated that Amcide is made by Nissan in Japan.

Just to put the record straight, Nissan Chemicals manufactures ammonium sulphamate but Amcide is the trade mark of Albright and Wilson Ltd, which markets ammonium sulphamate in the UK.

R. E. C. HAWKINS

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Albright and Wilson,
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Alastair Hay adds: The article on alternatives to 2,4,5-T also mentions criticisms by environmentalists of the widespread use of polychlorinated biphenyls. In it the phrase "polychlorinated biphenyls (PCBs) such as DDT" should have read "polychlorinated biphenyls (PCBs) as well as DDT". Dichlorodiphenyltrichloroethane (DDT) is not, of course, a polychlorinated biphenyl.

The November 25 issue reported Dr Donald Lee as saying that 130 kg of dioxin (TCDD) was released in the accident at Seveso. Dr Lee has since indicated that his suggestion was that "given the worst possible situations up to 130 kg TCDD could have been produced". His figure does not refer to the quantity of TCDD discharged directly into the atmosphere; as far as he is aware no figures for the amount of TCDD remaining in and around the reaction vessel have been divulged.

news and views

Parity violation in atoms?

from F. E. Close

Is parity violated in atomic physics? According to the experiments being performed independently at Oxford and the University of Washington the answer may well be no (this issue of *Nature*, page 528). This is a very interesting result in light of last month's report (Benvenuti *et al.*, *Phys. Rev. Lett.*, **37**, 1039; 1976) claiming that parity is violated in high energy "neutral current" interactions between neutrinos and matter.

The majority of theoretical high energy physicists currently believe that the weak and electromagnetic interactions are probably unified with the consequence that the atomic physics and high energy neutrino experiments should have shown similar behaviours with respect to parity. Consequently the atomic physicists' observations are highly significant and potentially dramatic for our understanding of Nature. To appreciate their relevance it is worthwhile surveying the developments of the past few years that have set the background for these experiments.

Unification of the weak and electromagnetic interactions

The unification of Nature's forces has long been the aim of physicists. In the 19th century Maxwell unified electricity and magnetism and in the past few years there has been an increasing belief among physicists that the familiar electromagnetic interaction may well be unified with the weak interaction. This latter mysterious force is responsible for β decay and is intimately linked with parity violation. If this unification idea is correct, then it would rank as one of the major advances in our understanding and hence much effort has been directed towards investigating it.

On the theoretical front there is the unified model of Weinberg (*Phys. Rev. Lett.*, **19**, 1264; 1967) and Salam (in *Elementary Particle Physics*, edit. by Svartholm N., 367, Stockholm, 1968). This idea was attractive but appeared to suffer from the difficulty that at high energies certain processes had infinite

probability. In the past few years there has been an upsurge of interest in this model as a result of three discoveries each of the first order of magnitude in significance.

In 1971 G. 't Hooft of the University of Utrecht showed that the theory was renormalisable, that is the infinities are no different from those that we are used to meeting in quantum electrodynamics and can be removed by traditional tools. This meant that for the first time one had a viable theory of weak interactions which did not violate any known general principle such as relativity, causality and unitarity and which, furthermore, suggested an intimate relation between weak and electromagnetic interactions.

On the experimental side was the discovery of "neutral current" interactions in which a neutrino interacts with matter without transfer of electrical charge, in contrast to the previously well known "charged current" interaction where the neutrino turns into a charged lepton (e^- or μ^-) with associated transfer of electrical charge to the target. That such neutral currents should exist was predicted in the Weinberg-Salam model and so their discovery was greeted with excitement (*Nature*, **245**, 119; 1973; **249**, 211 and **250**, 186; 1974). The charged current interactions have been known for many years to violate parity. The model predicted that the neutral currents should also be parity violating and this was confirmed by neutrino experiments only last month (Benvenuti, *op cit.*).

The discovery of neutral currents raised a problem, namely why is strangeness conserved in their interactions, in contrast to the charged current case? The theoretical answer was to postulate that a new family of particles should exist with a property called charm and with rather distinctive production and decay behaviours which have the effect of cancelling the unwanted strangeness-changing neutral currents (see *Nature*, **259**, 622; 1976). Two years ago this month came the

momentous discovery of the J/ψ particle (see *Nature*, **252**, 438, and **254**, 387; 1975) which led to the Nobel Prize for physics, being awarded jointly to B. Richter and S. Ting just a few weeks ago. This discovery was like finding a previously hidden window and the view through it led to the discovery this summer of the charmed particles. Consequently all the phenomena required for the unified theory to be valid were indeed being seen. Furthermore, by July of this year, a wide variety of data was being reported at the International Conference in Tbilisi USSR (see *Nature*, **262**, 537; 1976) in accord with the original unification idea of Salam and Weinberg.

Put all of these discoveries together and the excitement about unified theories of weak and electromagnetic interactions is natural.

Parity violation in atoms

According to the Weinberg-Salam model one should see parity-violating effects not only in $\nu \rightarrow \nu$ but also in processes involving electrons ($e^- \rightarrow e^-$) or muons. In these latter cases the parity-violating weak neutral current is swamped by the parity-conserving electromagnetic current; hence one would have to look for subtle effects. One such effect would be that a parity forbidden electric dipole transition could take place between atomic energy levels such as $1S_1$ and $2S_1$ in addition to the parity allowed magnetic dipole transition. It is effects associated with such small "wrong parity" admixtures (see Brodsky, S. and Karl, G., *Parity violation in atoms*, in *Comments on Atomic and Molecular Physics*, **5**, 63-69; 1976) that are being looked for by Sandar *et al.*

As these experiments currently stand, the predictions of the Weinberg-Salam model supplemented by atomic physics calculations appear inconsistent with the data.

As stated by both groups, their results are consistent with no parity violation. At present the discrepancy can conceivably be the combined effect

of systematic errors in atomic physics calculations and systematic uncertainties in the experiments. If, however, this state of affairs persists to a factor of ten below the present level and future high energy neutrino experiments (such as those about to begin at the CERN SPS in Geneva) continue to uphold the parity violation recently reported, then a rather paradoxical situation will have arisen in the light of currently favoured ideas.

The conclusion would probably be that neutrino interactions violate parity both when charge is transferred or when the neutral current process occurs. The parity-violating neutral

current with neutrino beams would have no obvious relation with electron beams which conserve parity. This is quite a tenable viewpoint—if one doesn't believe in unified theories! Alternatively, one might argue that there is some strange energy dependence whereby the high energy experiments show parity violation which is less marked at low energies (as in atomic physics). Whether such a possibility could be incorporated into the unification ideas is not clear. It also isn't clear, yet, if we have to worry. However, the clear blue sky of summer now has a cloud in it. We wait to see if it heralds a storm. □

about 30 tons is similarly driven through a 5.6 m rack and worm.

The azimuth and elevation positions are read into the computer from digital systems on the telescope axes and conventional drive systems give following accuracies on sidereal coordinates fed into the computer to ± 0.3 arc s. In actual operation the final guidance is by manual or automatic photoelectric corrections, on the object or offset star. These outputs are both fed into the computer and also operate quartz plates which adjust the position of the image. Experience has shown that the operational accuracy is one-fifth of the diameter of the image. That is, if the seeing disk is 1 arc s the guidance is accurate to 0.2 arc s. At the prime focus the manual setting system only is available but both manual and photoelectric devices are available at the Nasmyth foci. The controversial question of the problem of correction for image rotation in an alt-azimuth telescope appears to have been solved satisfactorily by these arrangements. In a 20 min exposure the errors in the correction for field rotation do not exceed 0.3 arc s. The slewing speed of the telescope is 45 deg min^{-1} in azimuth and 30 deg min^{-1} in elevation.

The finder telescope is of 70 cm aperture and objects of 17 mag are visible on the television display. Two fields of view, 30 arc min or 11 arc min, are available. At the prime focus guidance can be made directly or by offset on a 19 mag object. A television system for use at the prime focus is under development and it is expected that this will enable direct guidance to be carried out on objects to a limiting magnitude of +21.5 in blue colour.

The mirror

The 6 m mirror is of Pyrex glass. During the casting of the blank two small bubbles appeared and these spread somewhat during the grinding process. Small baffles in the region of the prime focus blank out these regions, but the consequent loss in intensity is only 10%. Otherwise the mirror has fulfilled expectations and although it will be replaced eventually by a mirror of SITALL material (the Soviet equivalent of CERVIT) there is understandably no great anxiety about the probable delay. The position on the availability of Soviet mirror blanks in SITALL is that blanks of 2 m are now available and the 6 m blank is expected to be available between 1979 and 1980 and the probable date for installation in the telescope is thought to be about 1983. The focal length of the 6 m mirror is 24 m giving a field of view at the prime focus of 2 arc min which can be increased to 20 arc min by the field correcting plates. The operating posi-

The Soviet 6 m telescope

from Bernard Lovell

It has been difficult in the West to obtain consistent information about the state of the Soviet 6 m optical telescope. It was therefore with particular interest that I received an invitation from the Soviet Academy of Sciences to visit the recently created Astrophysical Observatory, in the North Caucasus. The principal instruments of this observatory, which has the status of an Institute of the Academy, are the 600 m RATAN radio telescope and the 6 m optical telescope. A recent account of the RATAN radio telescope has been published (*Nature*, **262**, 535, 1976) and the following account deals mainly with the 6 m telescope as it existed in mid-October 1976 when I visited the observatory and talked with Dr I. M. Kopylov the director, Dr B. K. Ioannisiiani the chief designer and several members of the observatory staff.

Before choosing the site, I was told, fifteen areas in the Soviet Union were tested. It seems that an area in the Asian Republics might have been chosen but for the difficulty of transportation and constructing the telescope in such a region, and eventually the North Caucasus region was favoured. The telescope is 250 km from the airport of Mineralniye Vody, a 1 h 45 min jet flight from Moscow. The route rises slowly until the small town of Zelenchukskaya is reached 210 km from the airport and on the outskirts of this town the RATAN radio telescope has been constructed at an altitude of 1,000 m. From Zelenchukskaya the road continues normally for a further 24 km to where the new scientific village of Nizniy Azkhyz is

under construction. This village at present consists of a block of 70 flats for the scientists, a kindergarten, two shops and a cinema. The main scientific laboratory is under construction and more schools, a hotel and restaurant for visiting scientists are planned. From here an entirely new road 17 km long has been constructed to the telescope site which is at an altitude of 2,100 m in Stavropol territory, latitude $44^{\circ}44'N$, longitude $02^{\circ}40'E$. A small hostel in which we were housed provides a pleasant residence in beautiful surroundings about 800 m from the telescope. When the hotel in the village is complete this hostel will be used only by those using the telescope.

Design and guidance

The design of the telescope is unique in that it is the first large optical telescope to make the break from the conventional polar axis to an alt-azimuth mount. This has resulted in a most elegant construction and the system is clearly a great success. The rotating part of the telescope weighs 800 tons carried on six oil pad bearings and a locating but not load-bearing pedestal beneath. The vertical is maintained to 2 arc s by this system. The azimuth drive is through a 5.6 m gear and worm; the 5.6 m rack with 512 teeth was cut in one piece. This precision worm drive is protected from accidental overload by a spur gear and pinion, mounted immediately beneath it. If the load on the main drive rack reaches a specified level the worm is automatically shifted laterally so that the overload is taken on the subsidiary system. The elevation cage weighing

tions are either at the prime focus or at the two Nasmyth foci. The image scale is $0.12 \text{ mm arc s}^{-1}$ at the prime focus and $0.90 \text{ mm arc s}^{-1}$ at the Nasmyth foci.

Recording systems

In addition to direct photography two spectrographic cameras are available at the prime focus. Three gratings of 200, 300 and $600 \text{ lines mm}^{-1}$ and a selection of optical systems give dispersions in the range 60 to 370 Å mm^{-1} . One Nasmyth focus is occupied by equipment for stellar spectroscopy. The other focus houses an echelle spectrograph with two gratings. One with $100 \text{ lines mm}^{-1}$ giving a dispersion of 36 Å mm^{-1} in the range $3,200$ to $6,700 \text{ Å}$, and the second with 50 lines mm^{-1} giving a dispersion of 45 Å mm^{-1} in the range $6,500$ to $11,000 \text{ Å}$. The modern Kodak emulsions are used and up-to-date emulsion treatment facilities are available.

As regards electronic recording systems a one to three stage image tube system is working in the laboratory and will be tested at the prime focus before the end of November. Initially the spectrum will be recorded photographically but I was shown a digital recording system in an advanced state of development in the laboratory and was informed that this would be in use on the telescope in 1977. In this system real time sky subtraction will be available.

Sky conditions and sensitivity

The site has been carefully chosen to provide excellent seeing conditions. From the limited time for which the telescope has been in use the opinion is that 100 nights per year are absolutely clear and 200 nights about half clear, and for about 20% of the time the seeing conditions are 1 arc s. It is believed that temperature effects on the Pyrex mirror contribute significantly to this disk and that this 20% figure may be reduced to 0.5 arc s when the SITALL mirror is available. The present sensitivity of the system is that objects of +24 mag in blue colour with a signal-to-noise ratio of 3 to 5 are obtained with exposures of 30 to 40 min. Objects of magnitude +25 in blue appear with these exposures with a 64% probability of identification. In red the limit is +23 to +23.5 magnitude for a 90-min exposure. Dr Yu. Karovjakovsuy kindly presented me with a recent photograph of NGC 2419 obtained in blue colour with a 40 min exposure in which stars of +24 magnitude are clearly visible.

Contrary to the system favoured at Kitt Peak and Siding Spring the dome is effectively at ground level. Naturally the rotation is controlled by the telescope drive computer and the con-

struction, offices, laboratories and general facilities are of the highest quality. The telescope is already being visited by a considerable number of groups of the public. They are shown a film of the construction of the telescope in a lecture theatre inside the dome and can inspect the telescope through a viewing gallery. The telescope area itself is air conditioned and temperature controlled. The temperature is set to the predicted night temperature some hours before observations commence.

The chief designer of the telescope, Dr Ioannisiani, told me that the cost of the telescope itself together with associated equipment was about 30 million roubles. At current exchange rates this is of the order \$36 million, although it must be remembered that because of large variations in the cost of labour and materials the equivalence must be treated with caution. The cost of the dome, of the road up the mountain, the buildings at the observatory site and the erection of the new village, in addition to the cost

of the RATAN radio telescope implies that the Soviet Academy has invested a substantial sum of money in this new Institute. This courageous enterprise has given Soviet astronomers an unsurpassed instrument for the study of the heavens. The final tests and adjustments of the telescope by the observatory staff will be completed shortly and from January 1977 it is expected that the telescope will be in regular use for astronomical research. The instrument probably marks an historical epoch in the development of astronomy for two reasons, first because it is unlikely that a larger telescope will ever be constructed on Earth, and second because the success of the alt-azimuth mounting may lead to the abandonment of the polar axis design for any future large telescopes.

I wish to express my gratitude to the Academy of Sciences of the USSR for the invitation to visit this telescope and to Dr Ioannisiani, Dr Kopylov and many members of the observatory staff for their kind attention during my visit. □

Rethinking human evolution

from our Palaeoanthropology Correspondent

MANY areas of science occasionally make rapid advances when new data, new analytical tools and new areas of investigation suddenly open up new realms of perception, insight and knowledge. During such a phase the accumulation of data may proceed so rapidly that the older theoretical foundations to which these data are applied may require rethinking and revision. When that occurs it is necessary to step aside briefly in order to reassess those theoretical frameworks from the vantage of the new evidence. The study of fossil man has recently been undergoing such a period of saltatory expansion. The abundant new African material, from Lake Turkana (formerly Lake Rudolf), Laetolil and Hadar has accumulated so rapidly that there has been little time to reflect on how this material may affect current theories of evolution and our understanding of certain taxonomic categories.

Within the early years of this decade students of human evolution have already had to recognise two important and inter-related alterations in earlier theories of human emergence. The first of these concerned the evidence that two types of hominid had existed contemporaneously within Plio-Pleistocene times. Although some work had earlier

indicated such sympatry, the Lake Turkana and Hadar discoveries have provided virtually unquestionable evidence that at least two forms of hominid had coexisted (see Leakey and Walker, *Nature*, **261**, 572; 1976).

Such evidence indicates that the earlier and simpler models of "straight-line" evolution (for example, *Ramapithecus* → *Australopithecus* → *Homo*) could not be substantiated in the fossil record. Thus, the evolution of the Hominidae is more complex, and ultimately more interesting, than previous indications.

The second implication of the new fossil material is that the genus *Homo* may be far older than previously thought. A recent paper in *Nature* (M. Leakey, *et al*, **262**, 460; 1976) suggests the presence of *Homo* at Laetolil, Tanzania as early as 3.77 Myr ago. In that suggestion the authors have demonstrated, perhaps unwittingly, one of the more serious problems in studies of early man today. That problem is a lack of usable and valid definitions for genera within the family Hominidae. In that paper, the authors point out certain similarities between the jaws and teeth of the Laetolil hominids and others from such sites as Olduvai, Sterkfontein and Taung. Yet the Sterkfontein hominids have often been

placed within the gracile australopithecine group and Tobias has favoured placing the Taung child within the robust australopithecines. The acknowledgement of such morphological affinities does not strengthen the final attribution of the Laetolil hominids to the genus *Homo*.

However, the suggestion that this, or any material, belongs to the genus *Homo* should not rest solely on the presence of certain dental features. All hominids share a basic, underlying morphological pattern which is particularly evident in the jaws and teeth. The ultimate placement of fossils in the genus *Homo* must be based, first, on a clear statement of what characters constitute that genus and how it is distinguished from other contemporaneous hominid taxa.

Walker has recently (*Earliest Man and Environments in the Lake Rudolf Basin*, edit. by Coppens, *et al.*, Chicago University Press, 1976) taken a step in this direction by proposing a working model for a differential diagnosis between the Plio-Pleistocene hominid genera. Admittedly tentative, this model is of interest in that it focuses on a broad spectrum of cranial and facial features but avoids use of specific dental characters. Such a model could provide a useful point of departure for a broader and widely acceptable definition of early hominid genera. Ultimately, however, such a definition must also be based on character complexes of the post-cranium. For example, we are now in a position to begin discussing limb proportions, weight distributions in the lower limb and the structure of the hip and knee joints in the early hominids.

Walker's model also provides a warning concerning the problems inherent in basing attributions on single characters or on a tightly inter-dependent suite of characters. When the cranium of KNM ER 1470 was found in 1972 it was widely considered to have belonged to an early member of the genus *Homo*. This attribution was based almost entirely on its relatively large cranial capacity (about 775 cc) and on skull characters related to that size. Walker argues, however, that when the absolute size factor is de-emphasised the total morphology of this skull does not demonstrate clear affinities with *Homo*. Although Walker stops short of attributing the KNM ER 1470 cranium to *Australopithecus*, its position within *Homo* is clearly now uncertain. The taxonomic position of the Laetolil hominids must also be considered uncertain. Both, however, provide useful examples of some of the intrinsic problems in our current poorly defined taxonomic schemes. The large amount of newly available data should, hopefully, be put to good use in improving

our recognition of the distinctions between the early hominid groups. □

Mathematics and casinos

from Robert M. May

JAMES BOND fans will recall the climactic moment of the first book, *Casino Royale*, when the fate of Bond's mission hung on a single hand of chemin-de-fer. This two-person zero-sum game is one of several variants of the generic game baccarat, in which each player is dealt two face-down cards, and has the option to add one face-up card (the banker deciding last). The aim is to maximise the point count of the hand, modulo 10, with court cards counting as zero; thus 9 is the best count. The game is mathematically interesting in that non-trivial game theory concepts are appropriate. The complete minimax solution for chemin-de-fer with an infinite deck (or, equivalently, a finite deck sampled with replacement) was given by Kemeny and Snell (*Am. Math. Monthly*, **64**, 465; 1957), and shown by Karlin to be an example of a general result (*Mathematical Methods in Games, Programming and Economics*, Pergamon, London, 1959). The player's optimal strategy is deterministic in all cases but one: when his first two cards total 5, he should draw with probability 9/11 and stand with probability 2/11. The banker likewise acts deterministically unless his count is 6 and his opponent has not taken a third card, whereupon he draws with probability 859/2288 and stands with probability 1429/2288. When both players adhere to their minimax strategy, the banker has an average gain of 1.28% of the stake. In the heart-stopping hand, Le Chiffre held a count of 3, having just dealt Bond an exposed (third) 9 card, and both players agonised over his decision whether to draw a third card. As this was in 1953 or earlier, their ignorance may be pardoned. Kemeny and Snell's exact optimal strategy mandates that Le Chiffre draw (as he eventually did).

Before July 1, 1970, the British Gaming Act covering banker's games permitted any game so long as the "chances" in it were equally favourable to all players, including the banker. This fatuous legislation initiated some fascinating statistical studies (Downton, *J. R. Statist. Soc. A* **132**, 543; 1969; Downton and Holder, *loc. cit.*, **135**, 336; 1972; Holder and Downton, *loc. cit.*, **135**, 221; 1972; Downton and Lockwood, *loc. cit.*, **138**, 228; 1975 and **139**, in the press; 1976), as the "chances"

generated by games like baccarat and blackjack with finite decks are not readily amenable even to sophisticated mathematics in conjunction with fast computers. The new Gaming Act is more sensible. It allows four games (roulette, dice, baccarat, blackjack) to be played in licensed clubs, and defines the rules of play.

Downton and his colleagues have explored the mathematical properties of the four permitted games. Roulette and dice are games of pure chance, and are relatively simple to analyse, although some interesting features emerge (of which more below). For baccarat in its chemin-de-fer version, the banker's expected gain of 1.28% under optimal play with an infinite deck is independent of the player's strategy (Downton and Holder, *op. cit.*), so that this game is also effectively one of pure chance for the player. The use of a finite deck makes for vastly greater mathematical complexity (Downton and Lockwood, *op. cit.*). Depending on the size of the deck, measured in units of 52 card packs, and on the cards that have gone before, there are situations where the banker should randomise his decision when holding 3 and giving 8, holding 4 and giving 1, holding 5 and giving 4, holding 6 and giving 6, as well as the Kemeny and Snell case of holding 6 and giving no card (but Le Chiffre should still always draw when holding 3 and giving 9). The odds are but little affected by all the complications (the banker's expected gain with a three-pack deck is 1.31%), and Kemeny and Snell's strategy remains an excellent approximation with finite decks.

In blackjack the aim is to get close to a count of 21 (court cards counting as 10) without exceeding it. Subtle variations in the rules make for interesting differences in the player's strategies and in the odds (see the papers by Downton and Holder). The variant played in American casinos attracted much attention in the 1960s, when the perfect player was shown to enjoy an advantage of about 0.1% with a one-pack deck (Thorp, *Proc. natn. Acad. Sci. U.S.A.*, **47**, 110; 1961; *Rev. Int. Stat. Inst.*, **37**, 273; 1969; Epstein, *Theory of Gambling and Statistical Logic*; Academic Press, London, 1967). With a four-pack deck, the odds favour the bank. The version permitted in Britain under the current Gaming Act gives the bank a gain of 0.13% and 0.59% with one- and four-pack decks respectively. Casino pontoon, the variant played in most British midland clubs in the 1960s under the old Gaming Act, gives the banker an edge of 1.57% and 0.63% with one- and four-pack decks: notice here that, unlike blackjack, the bank's advantage de-

creases with increasing deck size. It is amusing that, misguided by the American experience, most midland clubs played with four-pack decks, thereby diminishing their advantage. Of course few players have the skill to achieve these odds, and the bank typically wins around 2–15% of the stake per hand. Downton has verified these figures in an extended series of undergraduate laboratory experiments.

This work has intrinsic interest. It can also be tortured into yielding two morals for ecologists. Downton notes that an interesting characteristic of any game of chance is the "gain ratio", defined as the bank's average gain per unit stake divided by the standard deviation: the gain ratio is the reciprocal of the coefficient of variation. Although all players are doomed to long-term losses, the smaller the gain ratio the higher the probability that a player will win (or, equivalently, will survive through) any one session. In roulette, gain ratios vary from 0.46% playing single members to 3.77% playing groups of 24; in dice or craps they range from 0.99 to 4.42%; in chemin-de-fer with best play the gain ratio is 1.34%. The significance of the gain ratio is illustrated by considering a casino which spins its roulette wheel 90 times per hour. Suppose 20 people each bet £1 on each spin over an 8-hour session. The bank's expected winnings are £390, but the standard deviation is £189 if all players bet on groups of 12 numbers, £706 if all bet on single numbers. This is a consequential difference. The processes whereby, in Reddingius's memorable phrase (*Oecologia*, 5, 240; 1970), plants and animals "gamble for existence" in an unpredictable world has many similarities with these games. The notion of "gain ratio" is likely to have fruitful applications in ecology.

Casinos and gamblers are usually ignorant of the mathematical structure of their games and strategies, which have, rather, evolved by an intense and highly motivated process of trial and error. In some respects they therefore invite analogies with biological processes of evolution by natural selection. This is particularly so for the way primitive societies may, or may not, have unconsciously "evolved" optimum strategies of resource management. The advantage of gambling games is that the empirically evolved strategies can be compared with the exact optimum ones. Chemin-de-fer provides a beautiful example: the optimum strategy is for the banker to vary his choice of drawing or standing only when he holds 6 and has given no card; most clubs have mandatory instructions to the dealer, and these used to be Crockford's drawing card which

allowed options in three cases, namely, holding 6 and giving no card, holding 5 and giving 4, and holding 3 and giving 9; since 1972 the instructions provided by the Casino Association of Great Britain allow the banker a choice in the latter two cases only. Although the differences are inconsequential, it is notable that the strategies chosen by the casinos were never the best, and have changed for the worse. Casino owners should read Downton's work. □

Surprises in the Magellanic Clouds

from M. G. Edmunds

THE research potential of the Magellanic Clouds—the two nearby galaxies easily visible to the naked eye in the Southern Hemisphere—is underlined by the surprising result of a recent investigation of a star cluster in the larger of the two Clouds. An Australian group (Gascoigne *et al.*, *Astrophys. J.*, 209, L25; 1976) have used the Anglo-Australian 3.9-m telescope to study NGC2209, one of the so-called "intermediate age" globular star clusters in the Clouds.

For stars it is notoriously difficult to assign a reliable age by appearance. But for a cluster of stars, the morphology of a plot of colours of the stars against intrinsic brightness can yield a fair guess. The method essentially works by looking for the highest mass (that is, brightest and bluest) dwarf stars which are only just beginning to evolve into giants. This "turn-off point" from the Main Sequence of dwarf stars can then be compared with computed models of stellar evolution to estimate an age.

In physical appearance a globular cluster has a characteristic spherical shape, held together by the mutual gravitational interaction of the stars. All globular clusters in our own Galaxy seem to have formed at the same time as, or not very much later than, the formation of the Galaxy itself—they are "old" objects. In the Magellanic Clouds there exist globular clusters which have almost certainly formed recently, as evidenced by the existence of bright young blue dwarfs which will live only a short time as dwarfs on the Main Sequence. The "intermediate age" clusters formed sometime between the young and old. Since there are only old clusters in our own Galaxy, these young and intermediate age Magellanic Cloud objects are of great interest. What is surprising is that the Aus-

tralian group find good evidence that the stars in the NGC2209 cluster have relatively few heavy elements ("metals") in them, only about one-sixteenth of the solar abundances, and yet they estimate the age of the cluster as a mere 8×10^8 yr compared with a probable age in excess of 10^{10} yr for the Large Cloud itself. The problem is that the interstellar medium in the main body of the Large Cloud, as determined by several independent investigations of the gaseous nebulae, is certainly not less than one-half solar. If the cluster were very much older, then its composition could be explained by saying it formed out of metal-poor gas, and that metal synthesis in the supernovae explosion of massive stars had enriched the interstellar medium since then. But here is a cluster which is apparently fairly recently formed, and should therefore reflect the present chemical composition of its precursor interstellar medium. It is true that the cluster is one of the most outlying—some 6° or 6 kpc away from the centre of the main body of the Large Cloud, but it is unlikely that the chemical composition of the interstellar medium varies so drastically across the Cloud. Perhaps an outlying cloud of gas somehow survived with very little star formation and consequent metal enrichment, until efficient star formation was suddenly induced.

The metal abundance of the cluster seems well demonstrated since the group used two independent methods. One method relies on the displacement of the locus of stars (relative to Galactic solar composition star clusters) on the colour/brightness plot, due to the weakness of absorption by metal lines in the blue spectral region. A second estimate comes from taking advantage of the light-gathering capacity of a large telescope and isolating just a few spectral lines in two of the stars by interference filters. A comparison of the line strength with that observed for stars in clusters of known composition yields a metal abundance estimate. Both methods agree that the cluster is considerably metal deficient.

The accurate aging of the cluster is more uncertain. The authors unfortunately do not quote error limits on their estimate. An inspection of their published data suggests that using their aging method, errors would not push the age back further than 2×10^9 yr—still young compared with old clusters and the probable age of the Clouds.

If the age of the cluster is confirmed in subsequent work, then considerable revision of our ideas about the chemical homogeneity of the interstellar medium in galaxies may be required. Investigation of other similar clusters in the Clouds will be awaited with interest. □

Salt tolerance in halophytes

from C. D. Field

In large areas of the world, the soil is too saline to support economic agriculture and more land becomes non-productive each year because of salt accumulation. So recent research into the ability of halophilic plants to survive in very saline environments has taken on a new importance.

The basis of the salt tolerance of halophilic plants is their ability to maintain and tolerate a high salt concentration in the cell. Since many halophytes show a positive growth response to salt in their environment (although they may also survive in a salt-free environment) it has been argued that as well as participating in the necessary osmotic adjustments, the high ion concentration may also be required by the plant for normal metabolic activity.

One possibility might be that the enzymes of halophilic plants, like those in salt-tolerant bacteria, require high salt concentrations for optimal activity. But this possibility seems to have been disproved by recent work (see Flowers, *Ion Transport in Plant Cells and*

Tissues, North Holland; 1975) which shows that *in vitro*, enzymes from halophytes are inhibited by ion concentrations much lower than those expected to occur in the cytoplasm, and very similar to those tolerated by enzymes from non-halophytes. Flowers has recently shown (*Proc. R. Soc. Lond.*, **B273**, 523; 1976) that malate dehydrogenase from the halophyte, *Suaeda maritima*, is activated at electrolyte concentrations similar to those required to activate the enzyme from non-halophytes. All the evidence therefore points to the conclusion that halophyte enzymes are neither particularly salt resistant nor salt requiring.

This leads to the idea that the excess sodium ions must be isolated from the cytoplasm in some way. Indirect evidence from efflux analysis and ion mobilities in tissues of halophytes suggests that sodium ions are retained in the vacuole (Flowers, *op. cit.*) and Jeschke and Stelter (*Planta*, **128**, 107; 1976) have shown that sodium ions are largely excluded from the cytoplasm of the halophyte *Atriplex* and barley, using flameless atomic absorption spectroscopy on thin slices of single roots.

This however raises enormous problems of osmotic imbalance between the

cytoplasm and the vacuole, which on this hypothesis would hold most of the salt accumulated in the cell. This osmotic imbalance could only be overcome if the water potential of the cytoplasm could be lowered, so that it will attract water from the vacuole and remain hydrated. This could theoretically be achieved in various ways. The cytoplasm might have an unusually high water-holding capacity arising from its structure, Donnan equilibria might exist between cytoplasm and vacuole, or a non-toxic osmoticum might accumulate in the cytoplasm. There is little evidence for the first two possibilities, but there is increasing support for the third.

Some years ago Stewart and Lee (*Planta*, **120**, 279; 1974) proposed the amino acid proline as the possible osmoticum. They found that in many halophytes proline accounts for more than 30% of the amino acid pool, whereas it is much less in glycophytes although the level in some species increases in response to salt or water stress. Stewart and Lee also showed that proline concentrations of up to 600 mol m^{-3} do not inhibit enzyme activity *in vitro*.

At a recent International Workshop on transmembrane ionic exchanges in plants (CNRS, Rouen, July 1976) Wyn Jones *et al.* put forward another candidate, the quaternary ammonium compound betaine, for the balancing osmoticum. They demonstrated that the betaine content of the halophytes *Atriplex spongiosa*, *Spartina x townsendii* and *Suaeda monoica* increased with external sodium chloride concentration, and that there was a close correlation between betaine accumulation and an increase in sap osmotic pressure above about 400 osmol m^{-3} . They also found that the *in vitro* activity of malate dehydrogenase was not inhibited by 1 M betaine, and that in the presence of low malate concentrations betaine provided partial protection to the enzyme against potassium chloride and sodium chloride toxicity.

These results suggest that betaine may have a similar role to proline in the cytoplasm of certain halophytes. Flowers has recently confirmed that similar levels of betaine occur in the halophyte *Suaeda maritima*. But in all cases direct experimental demonstration of betaine in the cytoplasm is still needed.

A simplified picture of a typical halophilic plant cell now emerges in which the potassium ion and sodium ion concentrations in the cytoplasm are of the order of 100 mol m^{-3} and 150 mol m^{-3} , whereas the vacuole has corresponding concentrations of 100 mol m^{-3} and 500 mol m^{-3} (Flowers *op. cit.*), and the osmotic balance in the cytoplasm is preserved by proline

Hormones, ducks and sex

from John Krebs

It is well known that steroid hormones influence sexual and aggressive behaviour in many vertebrates. These established and documented effects are of a rather long term nature, involving changes over developmental, seasonal and similar time periods. Recently, J. Balthazart (*J. Zool.*, **180**, 155; 1976) has suggested that much shorter term changes in behaviour might also be caused by fluctuations in hormone levels. He recorded the daily pattern of behavioural activities in two groups of five male domestic ducks housed in outdoor cages during winter. At the same time he took serial blood samples from each male to measure plasma testosterone.

The most interesting behaviour categories for the present discussion were courtship displays, mating attempts, and aggressive attacks on other males or females (there were two females in each group of males). Courtship and mating activity decreased through the day after an early morning peak, although some of the other activities did not show a similar daily rhythm, so there was not simply an increase in lethargy as the day progressed. In relating

these changes to plasma testosterone, Balthazart found that the hormone was at a higher level early in the morning than at other times of day, correlating well with the frequency of sexual behaviour. Further, some of the contrasts between the two groups were associated with hormonal differences. One group showed an increase in both display frequency and hormone levels in the afternoon while the other showed neither, but this second group had a higher level of sexual activity overall and a higher mean level of testosterone.

Behavioural differences between individual males did not relate clearly to hormone levels. There was a very slight tendency for males with higher testosterone levels to be more sexually active, but differences in daily rhythm did not relate to hormonal differences.

Balthazart's results raise an intriguing explanation for a mechanism of short term changes in behavioural activity, but it still has to be shown that the changes in hormone level cause the behavioural changes, rather than the other way round.

or betaine.

As with most models anomalies inevitably arise and in this case some of the marine algae raise pertinent questions. In *Valonia ventricosa* the cytoplasmic potassium level has been reported as of the order of 440 mol m^{-3} and the sodium level as about 40 mol m^{-3} whereas the corresponding vacuolar levels are about 620 mol m^{-3} and 40 mol m^{-3} (Gutknecht, *Biol. Bull.*, **130**, 331; 1966). The cytoplasmic potassium concentration in this instance is well above the demonstrated level for inhibition of enzymes from other halophytes. A re-examination of the ion concentrations in the various compartments of this particular alga is clearly indicated. At the same time it would be useful to find whether any metabolic adaptation such as adaptation of enzymes to salt has occurred or whether any compatible organic solutes are present in unexpectedly high quantities.

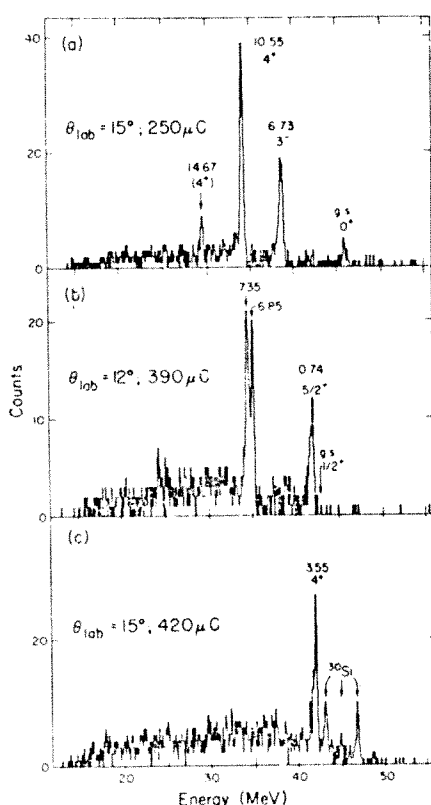
Nuclear spectroscopy with the (α , ^3He) reaction

from P. E. Hodgson

NUCLEON transfer reactions are one of the most powerful ways of determining the properties of nuclear states, and over the years very many studies have been made of practically every nucleus in the periodic table. The one-nucleon transfer reactions are particularly useful for determining the single-particle properties of nuclei, while reactions transferring more than one nucleon provide information about their collective properties.

For practical reasons not all nucleon transfer reactions are equally easy to carry out. For example, reactions with incoming or outgoing neutrons cannot be studied with the same energy resolution as those involving charged particles. Any addition to the list of reactions available for nuclear spectroscopy is therefore most welcome, and in recent years use has been made of reactions that have unstable outgoing particles.

One of the first of these unstable particles was the nucleus ^8Be , which rapidly decays into two alpha particles. The energy of breakup is however very small, only 96 keV, so the two alpha particles are emitted in almost the same direction, and are thus easily detected. Now another such particle, the nucleus ^3He , consisting of two protons in an $^1\text{S}_0$, $T=1$ state, has been shown by a group at Berkeley to be detectable in nuclear reactions. This is important because the (α , ^3He) reaction can now be studied, and this provides a convenient



Energy spectra of ^3He nuclei from the (α , ^3He) reaction on ^{12}C (a), ^{13}C (b), and ^{16}O (c).

way of transferring two neutrons to a nucleus.

In their preliminary studies (*Phys. Rev. Lett.*, **37**, 812; 1976) they show how the ^3He nuclei can be detected by a pair of proton counters. Almost as soon as it is emitted from a reaction, the ^3He nucleus breaks up into two protons, travelling in almost the same direction, and these can almost simultaneously activate two adjacent proton counters. The number of counts from two unrelated protons is very small.

They have used this detection system to study the (α , ^3He) reaction on ^{12}C , ^{13}C , and ^{16}O and find energy spectra very similar to those found for reactions with stable outgoing particles. It is therefore possible to use it to study the structure of the residual nuclei.

Some of their spectra are shown in the figure, and it is notable that very few states are excited, showing that the reaction is very selective. This means that the reaction will only go to states of a particular structure, and this enhances its value as a spectroscopic tool.

Examination of the states excited in the reactions mentioned shows that they are all of high spin. The final nuclei are ^{14}C , ^{15}C and ^{16}O and the states excited are the 3^- at 6.73 MeV and the 4^+ at 10.55 MeV in ^{14}C ; the $5/2^-$ at 0.74 MeV in ^{14}C ; the $5/2^-$ at 0.74 MeV in ^{15}C ; and the 4^+ at 3.55 MeV in ^{16}O .

The preferential excitation of high spin states has already been observed in

the (α , d) reaction and these states are favoured because the kinematics of the reaction are such that the neutron-proton pair is easily captured into a relative triplet state about an undisturbed target core. In the same way the observed selectivity in the (α , ^3He) reaction corresponds to the capture of a neutron-neutron pair into a relative singlet state. At 65 MeV, the energy of the alpha particles used in this experiment, the angular momentum transferred in a surface reaction is about four or five units of \hbar , so that states formed by capturing the two neutrons into d orbitals with the configuration $(d_{5/2}^2)_1^+$ should be preferentially excited. The known spins of the states that are selectively excited are in agreement with this simple picture. Similar arguments can be used to predict the states that will be excited in reactions at different energies and on different nuclei.

These results already show the usefulness of the (α , ^3He) reaction in exciting states of high spin in nuclei. It is likely to be applied to study the structure of many nuclei throughout the periodic table. □

Pressure solution

from K. R. McClay

The Tectonic Studies Group of the Geological Society held a discussion meeting at Imperial College on the subject of pressure solution and Coble Creep, on November 5, 1976.

THE role of diffusive mass transfer processes in geological deformations, although indisputably important, has always been difficult to assess critically and even more difficult to quantify. Ever since Sorby, in the late 19th century, realised that some rocks exhibit textures and structures that can only be explained by 'solution' at highly stressed grain boundaries and re-deposition of minerals in less stressed regions, structural geologists have been trying to understand this phenomenon of 'pressure solution'. This rock deformation mechanism has largely been ignored since the turn of the century, and only in the 1960s was interest in pressure solution revived, chiefly by J. Ramsay.

The meeting was intended to review current research on pressure solution and diffusion processes in rocks and also to evaluate their importance in geological deformations. Although the meeting was concerned with the fundamental aspects of pressure solution processes, these are also of considerable importance in the compaction behaviour of the reservoir rocks

in some oil fields.

Flow by diffusive mass transfer involves transport of matter from grain boundaries subjected to a high normal stress to grain boundaries which are less highly stressed. If the diffusion is predominantly through the grains, the process is termed Nabarro-Herring Creep, whereas if it is predominantly around grain boundaries, the flow is called Coble Creep. In general, grain boundary sliding must occur to accommodate the grain shape changes due to diffusion. These processes only proceed at a detectable rate, when the temperature is a large fraction of the absolute melting temperature of the material. Whereas processes involving solid state diffusion probably occur in high grade metamorphic rocks and in the mantle, geologists have long recognised that textures such as tectonic stylolites, truncated fossils and tectonic overgrowths in low grade metamorphic rocks often indicate deformation by diffusive mass transfer. Even over the relatively long time available for geological deformations, solid state diffusivities would be too slow to account for the observed strains. It is therefore inferred that the rate of diffusive mass transport is enhanced by the presence of an intergranular fluid film—hence the term pressure solution. Thus Coble Creep and pressure solution are geometrically similar in that they both involve intergranular diffusion.

E. Rutter (Imperial College) demonstrated that Coble Creep and pressure solution have similar flow laws (for a review see Rutter, *Phil. Trans. R. Soc. Lond.*, **A283**, 203; 1976), and indicated that the rate of diffusive mass transfer is generally considered to be the rate-controlling step during deformation. Rutter also emphasised the importance of recognising the several possible diffusion paths in low grade metamorphic rocks (R. Knipe, Imperial College). This research indicates that in some sandstones, intracrystalline diffusion along subgrain walls may be able to give rise to strain rates equivalent to those for pressure solution along grain boundaries. Stress relaxation experiments (E. Rutter and D. Mainprice, Imperial College), clearly showed the strong influence of pore water on the creep behaviour of Tennessee sandstone. They inferred that this behaviour indicates deformation by grain boundary sliding accommodated by diffusive mass transfer. In a review of the field evidence for pressure solution (such as striped cleavage, tectonic stylolites, and truncated fossils), J. Ramsay (University of Leeds) indicated that two types of pressure solution regimes occur; an isochemical regime where precipitation of the dissolved phase occurs close to the

generating interface (giving pressure shadows and overgrowths) and a regime where the bulk chemistry is changed because the dissolved material is precipitated elsewhere (giving tectonic veining). The influence of stress 'risers' which produce variations in the mean stress was considered important in the initiation of pressure solution seams.

M. Casey (University of Leeds) has formulated a purely solid state, Coble Creep finite element model for diffusion over several grain diameters (that is, Ramsay's second regime). Applying this to a study of differentiation in microfolds and crenulations he has concluded that fluid-assisted diffusion is necessary to account for these microstructures. B. Burton (CEGB Laboratories, Berkeley) discussed the available data on Coble Creep in metals and ceramics, and emphasised that this may not be an important deformation mechanism at low stresses and low homologous temperatures because of the stress needed to create sources and sinks for vacancy diffusion.

R. De Boer (Shell Laboratories, Rijswijk) pointed out the difficulties in experimental investigations of pressure solution. The process is intrinsically slow and cannot be speeded up because of the low heat of activation, and also the difficulty in suppressing plastic deformation and cracking.

In experimental simulation of oil reservoir phenomena, he has found that pressure solution phenomena occur in quartz sands above 280 °C and in carbonate mixtures at 200 °C. An important report of recent research involving fluid inclusion geothermometry and oxygen isotope studies was

given by R. Kerrich (University of Western Ontario). He suggested that grain size greatly influences pressure solution processes and that these processes are important in the deformation of fine-grained quartz and carbonate rocks at low metamorphic temperatures. A lively discussion was generated by the contribution of A. Beach (University of Liverpool) who considered that many of the residue minerals in pressure solution seams are in fact the products of prograde metamorphism. Metamorphic reactions could produce the silica found in the tectonic veins common in low grade metamorphic environments.

Over the past decade much data has been collected and many significant advances made, and there has been a convergence towards detailed microstructural, isotopic and chemical studies of natural pressure solution systems. Although only limited success has been achieved so far in experimental work, stress relaxation tests will allow access to the slow strain rates which are not attainable in normal triaxial tests. Grain size effects are very important and careful consideration of the various possible diffusion paths must be made. Many questions still remain unanswered, however, particularly about the origin of the periodicity of pressure solution seams and striping. Many research programmes are being pursued however, and these will undoubtedly produce results which will not only be of great academic interest but also of significance in the interpretation of some fault behaviour, and compaction behaviour of oil field reservoir rocks. □



A hundred years ago

WE frequently saw table-topped icebergs with the upper surface very irregular; when that is the case evidence may usually be found from the colour, the closeness of the veining and other appearances that it is not the original surface of the iceberg which is now presented to us, but a second surface produced by cutting away by the sea of an entire story, as it were,

of the berg; which although it had no doubt at one time during the process been greatly inclined, had recovered its equilibrium on the whole of the upper layer having been more or less symmetrically removed. (Conditions in the Antarctic: from a lecture given by Sir Wyville Thomson in Glasgow.) From *Nature*, **15**, December 7, 120; 1876.

review article

The Star of Bethlehem

David W. Hughes*

The Star of Bethlehem was probably a triple conjunction of Saturn and Jupiter in the constellation of Pisces, the significance of which was only obvious to the Magi of Babylonia. This occurred in 7 BC and events indicate that Jesus Christ was probably born in the Autumn of that year, around October, 7 BC.

THE Messianic star which heralded the birth of Jesus Christ and shone in the skies over Bethlehem, Judaea has been a phenomenon of considerable interest and much mystification for centuries. Almost every Christmas the star is pondered over and discussed. Invariably the same conclusions are drawn—the cause and form of the star are still uncertain. The astrologically significant triple conjunction of Saturn and Jupiter in Pisces is the most likely candidate but the two comets of March, 5 BC and April, 4 BC cannot be dismissed. Neither can the possibility that the star was simply legendary.

Biblical background

The principal account of the star is found in the gospel of St Matthew. It begins (Matthew II, 1–2):

"Now when Jesus was born in Bethlehem of Judaea in the days of Herod the King, behold, there came wise men from the east to Jerusalem, saying, where is he that is born King of the Jews? For we have seen his star in the east, and are come to worship him."

The term "in the east" in the second verse is incorrect in this Authorised Version translation^{1,2}. Originally it was written *en té anatólē* (Greek singular) whereas "the east" is usually *anatólai* (Greek plural). *Anatólē* has a special astronomical significance, indicating the earliest visible rising of a star at day break (the helical rising), and so the second verse should read "for we have seen his star appear in the first rays of dawn". These first two verses also indicate that Jesus was born and the visit of the wise men took place some time between 39 and 4 BC when King Herod was on the throne. Learning of the prophecy (Micah V, 2), "But thou, Bethlehem Ephratah, though thou be little among the thousands of Judah, yet out of thee shall he come forth unto me that is to be ruler in Israel.", Herod told the wise men that this is where Christ would be.

"Then Herod, when he had privily called the wise men, enquired of them diligently what time the star appeared. And he sent them to Bethlehem and said, Go and search diligently for the young child; and when ye have found him bring me word again, that I may come and worship him also. When they had heard the king, they departed; and lo, the star, which they saw in the east went before them, till it came and stood over where the young child was. When they saw the star, they rejoiced with exceeding great joy. And when they were come into the house, they saw the young child with Mary his mother and fell down, and worshipped him: and when they opened their treasures they presented unto him gifts; gold and frankincense and myrrh."

This second passage contains many interesting points. Very few people saw the star. Although the wise men saw it, Herod and

all Jerusalem did not, so the Christmas card image of a huge brilliant star illuminating all Bethlehem cannot be correct. It seems also that there were two separate appearances of the star. The first induced the wise men to leave the east and set out for Judaea; they then lost sight of it for a time. The second occurred when they were in Jerusalem and here it pointed out to them the place at Bethlehem where the object of their search was to be found. Not only did it point out Bethlehem, but it "went before them" and finally "stood over" Bethlehem. The star must have then been in the zenith. This ties in with the legend recounted by Maunder³. Apparently the star had been lost in the daylight by the time the wise men reached Jerusalem (indicating that it was an evening star during their journey). On reaching Bethlehem, apparently nearly at midday, one of them went to the well of the inn to draw water and, on looking down the well, saw the star reflected in the surface of the water—again indicating that it was in the zenith and that they had arrived at the right place. (For a discussion of seeing stars in wells during the day time, see refs 4 and 5.) It must be noted, however, that if a star is in the zenith it will appear to be equally over every object in the neighbourhood.

A star had been mentally associated with the coming of the Messiah for a long time. The oracle of Balaam (Numbers XXIV, 17) said,

"I see him, but not now;
I behold him, but not nigh:
a star shall come forth out of Jacob and a sceptre shall rise out of Israel."

This idea is, in fact, attributed to a man whose home was near the river (Numbers XXII, 5), that is in Mesopotamia, where there was great interest in astrology and astronomical objects and where the Old Testament would be well known and substantively the same as it is now. Some present-day scholars⁶ however think the term "star" in Numbers (XXIV, 17) applies to the Messiah himself and not to the sidereal phenomenon heralding His appearance.

The third reference to the star is not in the Authorised Version of the Bible but in one of the infancy gospels that were omitted when the Bible was compiled. The Protoevangelium of James (XXI, 2) states⁷:

"And he (Herod) questioned the wise men and said to them: 'What sign did you see concerning the new born King?' And the wise men said: 'We saw how an undescribably great star shone among these stars and dimmed them, so that they no longer shone, and so we knew that a King was born for Israel. And we have come to worship him', and Herod said: 'Go and seek and when you have found him, tell me, that I may also come to worship him'. And the wise men went forth. And behold, the star which they had seen in the east went before them, until they came to the cave. And it stood over the head of the child."

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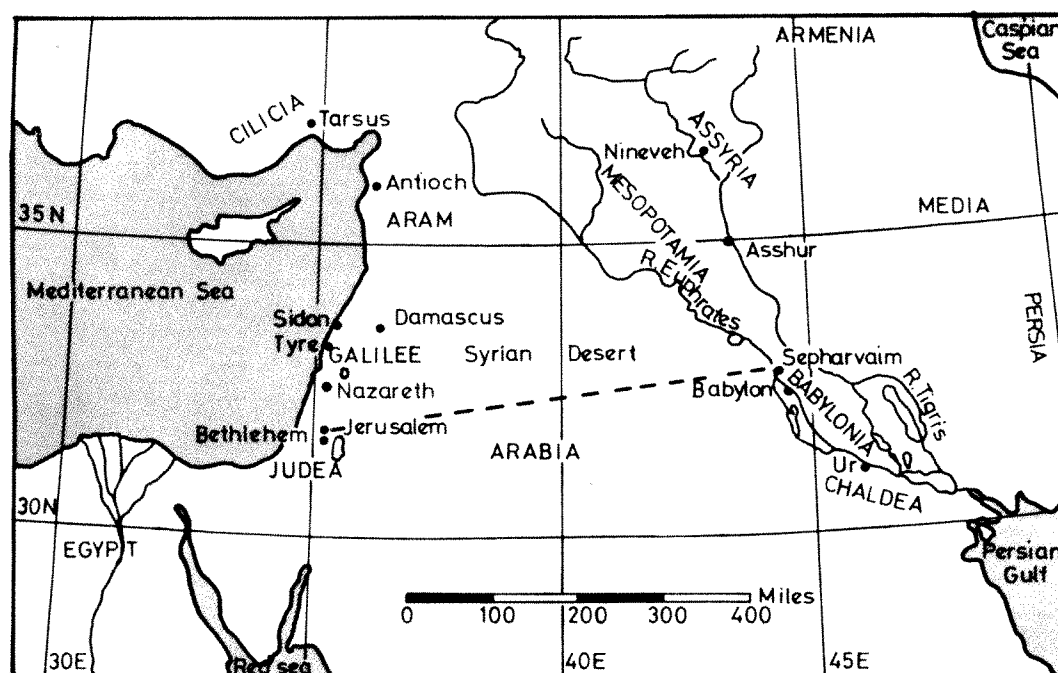


Fig. 1 The Middle East indicating one of the many possible routes of the Magi's journey from the east to Jerusalem and Bethlehem.

Here clues are given as to the star's brightness. To dim the other stars requires it to have a brightness comparable with or even brighter than the full Moon. But if this was so why didn't Herod and all Jerusalem know of it?

The wise men

The word Magi comes from the greek (μαγοι) which the Authorised Version translated as "wise men" and the *New English Bible* as "astrologers". They were probably Median priests of Zoroastrianism who, apart from performing the duties of national priesthood, used to occupy themselves with the interpretation of dreams. Babylon, Assyria and Chaldea have been put forward as their place of origin (Fig. 1). It has also been suggested that Magi does not refer to a specific race at all but is just a general name for a priestly cast of "magical" tendencies. In the earliest carvings and pictures they are shown in Persian dress, wearing trousers. It is said that such a carving saved the Church of the Nativity of Ravenna from the general havoc of the Persian onslaught in AD 614. Astrology, the prediction of future events from the positional relationship between the Sun, Moon and planets and the stellar background, was definitely one of their practices.

In the language of the Old Testament and probably St Matthew as well the term "the east" was very vague and included countries that lay considerably to the north as well as the east of Palestine. Previous biblical books (Isaiah LX, 6; Psalms LXXII, 10, 15) predicted that the kings would come from Arabia (Sheba, Seba and Tarshish). But other biblical scholars have favoured Persia, Parthia (a country south-east of the Caspian that used to extend from the Indus to the Euphrates) and Babylonia. Wherever they came from they would have been expecting that a King of the Jews was to be born and have probably learnt of this from Jewish sources. (This expectation was widespread, compare Simeon in Luke II, 25.) The nature of the gifts does not present a clue.

It was not until the sixth century that tradition changed the Magi into kings. Their number was fixed at three because of the threefold nature of their gifts (eastern tradition has it that there were twelve).

The date

In AD 525 Dionysius Exiguus, a prominent scholar and Roman monk, fixed the origin of the present calendar so that Jesus was born in December, AD 1 (anno domini, "in the year of the Lord"). AD 1 corresponded to AUC 754 in the Varronian

reckoning (AUC standing for *ab urbe condita*, from the founding of Rome). Unfortunately Exiguus forgot the year zero which should have been inserted between 1 BC and AD 1 (in fact astronomical reckoning has AD 1 = 1, 1 BC = 0, 2 BC = -1 and so on) and also the 4 yr when Emperor Augustus ruled under his own name of Octavian. So Exiguus was at least 5 yr out.

Jesus was born in the days of King Herod. According to the historian Flavius Josephus⁸, Herod died within a few days of an eclipse of the Moon visible from Jericho and a few days preceding the feast of unleavened bread. The eclipse is the only one mentioned by Josephus⁹ and must have been that which occurred during the night of March 12-13, 4 BC. Passover started on April 11, so Herod died between March 12 and April 11, 4 BC. Christ must have been born before this. In fact when Herod died Christ was in Egypt with his parents, the family having taken refuge there after being warned of Herod's impending wrath.

On realising that the Magi had not returned to Jerusalem to tell him where the child was, but had gone back to their country another way, Herod sent men to kill all the children in Bethlehem and its environs who were 2 yr old or less "according to the time which he had diligently enquired after the wise men" (Matthew II, 16), so Christ could have been anything up to 2 yr old at this time.

Luke II provides another clue about the birth date. Joseph and Mary journeyed to Bethlehem from Nazareth because Caesar Augustus decreed that all the world should be taxed. An ancient inscription unearthed in Ankara¹⁰ lists the years in which orders were issued for tax collection. The most feasible date is 8 BC, but slow travel and communications in those days could have delayed the actual collection of taxes by a year or two. Luke also states that this taxing was first made when Cyrenius (Quirinius in Latin) was governor of Syria. Quirinius was a consul in 12 BC and also fought against the Homanadensians in Cilicia before 6 BC. Tertullian¹¹ states that the census at the time of the birth of Jesus was taken by Sentius Saturninus who was governor of Syria between 9 and 6 BC. Quirinius could have been associated with Saturninus in this project some time between 6 and 5 BC, when he was an Emperor's legate in Syria. But he did not become governor of Syria until AD 6 so Luke was mistaken (II, 2). Needless to say if Luke were correct in II, 2, Christ would have been born some time between AD 6 and 7 when Quirinius governed Syria.

Some early Christian writers (Origen and Eusebius, for

example) state that Jesus was 2 yr old when the wise men came; he was then taken to Egypt and remained in Egypt for 2 yr before returning to Nazareth. St Luke mentions neither star nor Magi in his gospel but tells of the first month or so of Christ's life, of His circumcision after 8 d and of His being brought to the temple in Jerusalem after Mary's purification.

All in all the above facts indicate that Jesus was born in 6 bc but some biblical scholars, with justification, plump for 7 bc and 5 bc. A brief chronology is shown in Fig. 2.

On which day of the year was Christ born? Christmas Day, December 25, is celebrated all over the world as the birthday of Jesus but this date has only been used since about AD 336 (ref. 12). In those times this date was accepted as the time of the winter solstice, midwinter's day, after which daylength increases. The pagan feast of *dies solis invicti natalis* (the birthday of the unconquered Sun) was celebrated on that day, which occurred near the middle of Saturnalia, a season during which work ceased, houses were decorated with laurels and evergreens, gifts were exchanged and parties and processions were held. As many early Christians would not give up this pagan holiday the western Church decided to transform the pagan ceremony into a Christian festival by having Christmas Day on December 25. In the east the birth was celebrated on January 6, a date to which also the star and the Magi, the baptism of Christ and the miracle at the wedding at Cana were attached. January 6 was also the date of the festival in the temple of Kore at Alexandria and at places in Arabia, celebrating Kore, the virgin, giving birth to Aion. Again the same date could have been chosen to replace a pagan ceremony by a Christian one. Epiphanius (AD 315-403) gives January 6, AUC 752 (2 bc) as the birthday.

Clement of Alexandria in the *Stromata* (AD 194) gives November 18, 3 bc but also states that others think the birthday was either May 20 or April 19/20. Epiphanius, 120 yr later, stated that May 20 (or May 21 or June 20) were the dates of the conception and not the birth so some confusion exists here. However the dates were fixed, they seem to agree with an old

tradition that Christ was conceived in the spring and born around midwinter. Keller² introduces another interesting complication. According to St Luke (II, 8), "there were in the same country shepherds abiding in the field, keeping watch over their flock by night". The climatic conditions in what was then Palestine during Christmas time are most inclement, there being an average 6-8 inches of rain during December and January. Bethlehem is also in the grip of frost during December, January and February and no sheep would be in the fields. Flocks are usually put out to grass between March and November, the shepherds being with the flocks during the lambing season in the spring (March and April).

The star

The star must have been a fairly long lasting phenomenon, to "go before" and "stand over", which rules out transient phenomena such as fireballs and very bright shooting stars.

What of the more lasting transients such as comets and novae (discounting the fact that a brilliant comet or nova would have been seen by Herod who, according to Matthew and James, had not seen anything)? Halley's comet was first seen in 240 bc and owing to its 76-yr periodicity, reappeared in 12 bc. It was first seen about August 25 near Mu Geminorum and moved by Beta Leonis, disappearing in Scorpio about 60 d later. It was seen all over China but no report of its observation in the west has come to light. This comet is No. 51 in Williams' catalogue¹³ and is too early to be the Star of Bethlehem. No. 54 (of AD 13) is too late. This leaves No. 52 which appeared in March, 5 bc in the constellation of Capricorn and lasted 70 d, and No. 53 which appeared in April, 4 bc in Aquila and was possibly a nova being referred to in the Chinese as a "comet without a tail".

Some people think that Venus was "the star" and at the latitude of Bethlehem Venus is high enough in the sky to be a most impressive sight, being at times 15 times brighter than Sirius, the brightest star. But it is most unlikely that the Magi,

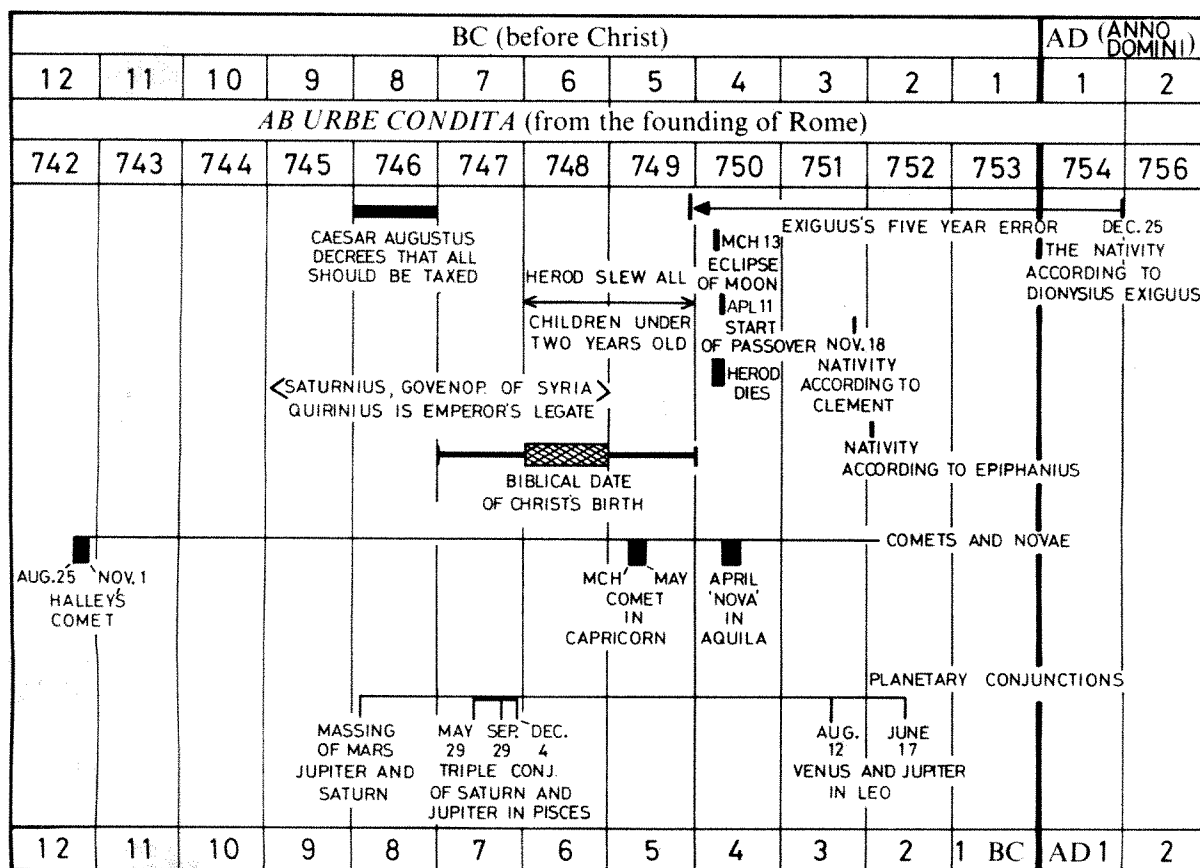


Fig. 2 A chronology of the major biblical and astronomical events occurring around the time of the birth of Christ.

constant observers of the sky, would be caught unawares by an appearance of Venus.

Another possibility is that the star was simply the conjunction between two or three planets. Two or more bright planets very close together in the sky provide a most striking sight. Johannes Kepler was fascinated by the conjunction between Jupiter and Saturn on December 17, AD 1603. In the spring of 1604 Mars moved into the vicinity of the other two and in autumn 1604 a supernova (SN 1604 Ophiuchi) appeared nearby. All in all, Jupiter and Saturn came into conjunction three times in 6 months. Jupiter and Saturn have orbital periodicities of 12 and 29 yr, respectively, so Jupiter on average passes Saturn every 20 yr. About every 120 yr, three successive conjunctions take place over about 6 months, this being known as a triple or great conjunction. Such a conjunction will occur every 120 yr and a similar one would have occurred in 7 BC. Pritchard^{14,15} confirmed this fact, and on May 29, September 29 and December 4, 7 BC a conjunction occurred in the constellation of Pisces, which is astrologically associated with the Jewish people. In February, 6 BC Mars moved into the configuration and formed an equilateral triangle with the other two planets, a situation known as a massing of planets¹⁶, but the Sun was too close for this to be observed with the naked eye.

Pritchard¹⁵ points out that in 66 BC, about two revolutions of Saturn and five of Jupiter before 7 BC, a closer single conjunction of the two planets took place, again in Pisces. In February, 66 BC the planets would set about 45 min after the Sun and would be less than 55 min of arc apart. Whether the astrological significance to the Magi of this single conjunction was the same as the significance of a triple conjunction is unknown. Maybe these previous conjunctions also impelled Magi to journey to Jerusalem, but the Bible only reported the fruitful journey.

Sinnott¹⁷ studied the 200 conjunctions and 20 multiple groupings that occurred between 12 BC and AD 70, these being gleaned from Tuckermann's book¹⁸. Sinnott concentrated on very close groupings, "weeding out" commonplace conjunctions and ones which were widely separated, and lists six conjunctions which could be seen from the Middle East in which the stars were less than 12 min of arc apart and more than 15° from the Sun (so they easily could be seen at night). These are shown in Fig. 2. Unfortunately none occurred between 12 BC and 3 BC, indicating (if the above thoughts about when Christ was born are correct) that Sinnott's selection criteria are too strict. The two conjunctions of the brightest planets, Venus and Jupiter, the first on August 12, 3 BC, visible in the eastern dawn sky from about 0345 h to 0520 h when they were separated by about 12 min of arc (in Leo, near Regulus) and a similar event on June 17, 2 BC when they were less than 4 min of arc apart and close to the western horizon where they would seem to fuse into one star "gleaming like a great beacon over Judaea to the west", would have been most impressive but do occur after Herod's death. The second conjunction again occurred near Regulus, a royal star. (Astrologically it is interesting that Judah, one of the tribes of Israel, has the lion, symbolising the constellation Leo, on its standard. Bethlehem, in the land of Judah, was the predicted birthplace of a "Governor that shall rule my people Israel" (Matthew II, 6). These facts were well known to the Magi.)

Possible sequences of events

It must be remembered that the Magi were skilled astrologers and accomplished star gazers with great knowledge of the sky, backed up by about 4,000 yr of astronomical observations. Many towns in the Middle East have legends retelling how theirs was the town from which the Magi started on their journey (the people of Saveh in Persia told Marco Polo that their town was the starting point). The triple conjunction of 7 BC had been predicted. The calculations and predictions are given in an almanac found on a cuneiform tablet at Sippar (Sepharvaim) in Babylonia, a town noted for its school of

astrology. The conjunction took place in Pisces, a constellation which is astrologically associated with the Jewish people and Israel (compare the writings of Rabbi Arbarband 1437–1508). The Sun moves through Pisces at the boundary between winter and spring and astrologically this indicates the end of an old age and the start of a new one. Jupiter was considered to be a lucky and royal star and in traditional Jewish astrology Saturn protected Israel.

Babylonian astrologers steeped in Jewish tradition would have predicted the triple conjunction in Pisces. Realising its astrological significance (that is, a Jewish king born in Israel) and remembering the prophecy of Balaam (Numbers XXIV, 17) and seeing in the sky their astronomical prediction come true would be ample justification for starting a journey to pay homage to the new king, the herald of a new age.

The journey from Sepharvaim to Jerusalem across the Arabian desert is about 550 miles. Preparing for and taking this journey would take about 4 months. The planetary clustering would have begun about the end of February, 7 BC with Jupiter moving from Aquarius towards Saturn in Pisces. As the Sun was also in Pisces at this time the planets would not be visible. Not until April 12 did both planets rise helically in Pisces (compare Matthew II, 2). The first close encounter (0.98 degrees separation—twice the diameter of the full Moon) took place on May 29 and was visible for about 2 h in the morning sky. The Magi could have set out towards the end of June, 7 BC, the September 29 conjunction confirming their predictions as they were nearing Jerusalem and the December 4 conjunction, occurring after the audience with Herod, pointing the way south to Bethlehem 5 miles away. Pisces rises at about 0200 h, 2200 h, 1900 h and 1200 h during May, July, September and December, indicating that the two planets would be clearly visible during their journey. The planets are due south (and closest to the zenith) about midnight during September and at about 1900 h during early December. (This throws doubt on to the Maunder legend of the midday star in the well.) Jesus could have been born about 2 months before the last conjunction, say in early October, 7 BC. He would have been circumcised at 8 d, then after the 33 d of Mary's purification taken to Jerusalem where a sacrifice was made. After returning to Bethlehem he was visited by the Magi. (The word *τοπαῖδν* in Matthew II, 11 means "a very young child" or infant.) After being warned in a dream about Herod's impending wrath, Joseph fled with Mary and Jesus to Egypt. In early October the shepherds could be moving the sheep from the hills to their winter quarters. Jesus would have been 2.5 yr old when Herod died.

A second scenario could be as follows. The conjunctions of 7 BC could simply have attracted the wise men's attention towards Palestine, the comet of March, 5 BC starting them on their journey. They would have arrived well before Herod's death (which occurred between March 12 and April 11, 4 BC) and being told that Jesus was born in Bethlehem, arrived there about April, 4 BC when the nova was shining. Before Herod died he ordered that all children under 2 yr old be killed, and with this scenario the order would have gone out just before he died, giving Joseph, Mary and Jesus time to flee to Egypt.

Jesus would have been about 2 yr old when this order went out and when seen by the wise men. This ties in with Epiphanius* who stated that the star shone in the east 2 yr before the Magi arrived in Jerusalem. So Jesus would have been born in the spring of 6 BC, Epiphanius's star could have been the conjunction and the shepherds would have been in the fields at lambing time.

But why should Joseph and Mary stay in Bethlehem for 2 yr when they had only gone there to be taxed? And why had not Herod and the Jews connected the comet of 5 BC with the coming of Christ? It is possible that Herod missed the triple conjunction when the planets only just came within 1° of each other. The Protoevangelium of James supports this latter picture.

Non-astronomical possibilities

One easy way out of the dilemma introduced by the Star of Bethlehem is to regard it as another miracle, as the direct intervention of the hand of God and thus as a phenomenon not requiring a scientific explanation.

It could also just be a legend. No king worth his salt in those days was born without some celestial manifestation. A star greeted the birth of Mithridates (131–63 BC) and Alexander Severus¹⁹. The temple of Diana at Ephesus was burned down the night that Alexander the Great was born, Magi announcing that the plague and bane of Asia had been born that night. The magos Tiridates and some followers came to Rome to acknowledge the divinity of Nero in AD 66.

Matthew, who wrote his gospel for a Jewish audience and who frequently cited passages of the Old Testament to prove points to his readers, probably felt that a star was necessary to fulfil the prophecy of Balaam. The Essenes, who studied the prophets to find interpretations of the contemporary scene, would believe that such a prophecy could not have been without fulfilment. They would go so far as to say: no star, no messiah. The absence of the Matthean clause "that it might be fulfilled" is very worrying and casts doubts as to how far the evangelist regarded the account of the star as historical. Albright and

Mann¹ suggest that the clause might have been "edited out" of Matthew's gospel at a later date to appease Gnostic opposition to the church. (The gospel of St Matthew was probably written some time between AD 65 and AD 85.)

Bearing in mind Herod's surprise on being told of the star by the Magi, the triple conjunction in 7 BC seems to be the most probable candidate. If this is so Christ would have been born in about October of that year.

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articles

Nuclease cleavage of chromatin at 100-nucleotide pair intervals

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Digestion of mouse liver nuclei with DNase II leads to a novel cleavage pattern with a 100-nucleotide pair periodicity. From chromatin, this pattern or the standard 200-nucleotide pair repeat can be produced depending on the ionic conditions. The results are interpreted by assuming different conformational states of the nuclear material, including condensed and extended forms.

THE discovery of a chromatin subunit has had a great impact on the concept of the structure of chromatin (for reviews see refs 1–3). Evidence for this subunit, which has also been termed ν -body or nucleosome rests mainly on results obtained from nuclease digestion of nuclei and on direct visualisation of particulate structures in the electron microscope. Approximately 200 nucleotide pairs of DNA seem to be wrapped around a globular histone bead which has been proposed to consist of two of each of the histones H2A, H2B, H3 and H4. Digestion of nuclei with an endogenous nuclease or micrococcal nuclease yields protected DNA fragments of 200 nucleotide pairs and multiples thereof, presumably by attack in a region between the subunits. Within the nucleosome, cleavage at preferred sites has been reported for micrococcal nuclease¹ and for DNase I which introduces single-strand nicks at intervals of ten nucleotides into the nucleosomal DNA².

We have studied various aspects of chromatin structure with the help of restriction nucleases³ and by a detailed investigation of the action of micrococcal nuclease^{7,8}. It seemed promising to extend the work to DNase II which has already been used in a few laboratories to digest chromatin^{9–11}. We have used this nuclease for several reasons: it introduces into DNA single-strand as well as double-strand breaks¹², it has no requirement for divalent cations in contrast to all other nucleases used for the digestion of chromatin, and even though its pH optimum is around 5, it retains sufficient activity at neutral pH to permit its use in physiological conditions¹². One unexpected outcome of our experiments was the finding that DNase II can cleave the DNA in chromatin and nuclei with a previously unobserved 100-nucleotide pair periodicity.

100-Nucleotide pair repeat pattern from mouse nuclei

Mouse liver nuclei were treated with DNase II and the DNA was analysed by Agarose gel electrophoresis. The resulting pattern is shown in Fig. 1, together with the pattern found with micrococcal nuclease in the same conditions. The positions of the peak maxima in the DNase II pattern correspond to sizes which are multiples of 100 nucleotide pairs (Fig. 1B), that is half the value obtained with micrococcal nuclease. The gradual shortening

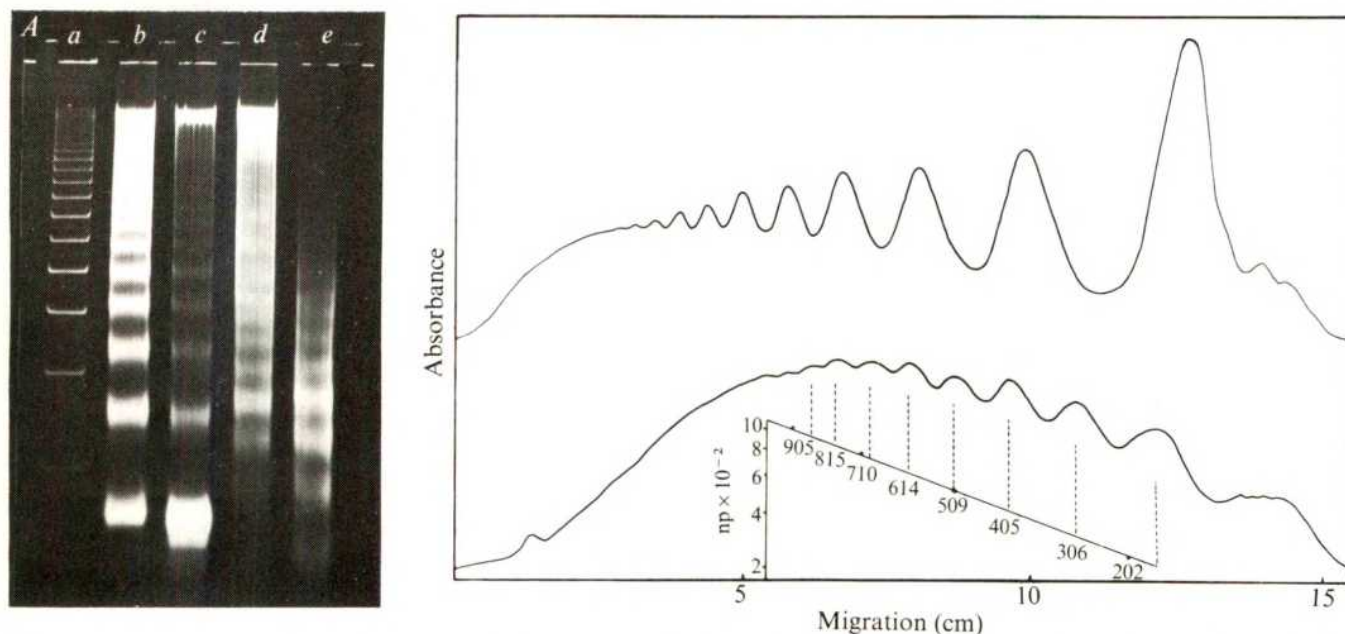


Fig. 1 Digestion of mouse liver nuclei with DNase II and micrococcal nuclease. Mouse liver nuclei were prepared as described before⁶. Samples containing 100 μ g of DNA were suspended in 10 mM Tris-HCl, pH 7.0, 1 mM phenylmethane sulphonyl fluoride (PMSF) in a total volume of 0.2 ml. Microscopic inspection showed the nuclei to be largely intact in these conditions. **A**, Incubations were for 1 h at 37 °C. In (b) and (c) micrococcal nuclease (Worthington) was present at concentrations of 7 and 25 U ml⁻¹, respectively, and the incubation buffer was supplemented with 1 mM CaCl₂. In (d) and (e) DNase II (Worthington) was added to concentrations of 125 and 250 U ml⁻¹, respectively. Slot (a) contains a mouse satellite DNA marker partially digested with *Eco*RII¹³. After incubation, DNA was extracted as described before⁶ and 5 μ g was applied to a 1.8% vertical Agarose slab gel, run in the Tris-borate buffer system as described before¹⁴. It was afterwards stained with ethidium bromide (1 μ g ml⁻¹) for 30 min. **B**, DNA from nuclei incubated with DNase II (250 U ml⁻¹) as in (A) was electrophoresed in a 1.8% Agarose gel with and without admixed mouse satellite DNA, partially digested with *Eco*RII. The gel was stained with ethidium bromide and photographed, and the negative was scanned as described before⁶. The lower tracing shows the DNase II pattern: in the upper tracing DNA from a micrococcal digest of nuclei is shown for comparison. Inset, average sizes of the DNase II fragments are depicted as they are determined in the coelectrophoresis with the satellite DNA digest. The molecular weights of the satellite fragments had been determined (R. E. Streeck, to be published) relative to the size of the sequenced restriction nuclease fragment H of SV40 virus (ref. 15 and W. Fiers, personal communication).

of the DNA fragments during digestion with micrococcal nuclease was not observed with DNase II. This may explain why the fragments in the DNase II digests seem to be slightly larger than the corresponding fragments from micrococcal nuclease digests (Fig. 1). A kinetic analysis of DNase II digestion (Fig. 2) indicates that the 100-nucleotide pair repeat is present as soon as discrete bands can be resolved, and persists throughout digestion.

To rule out the possibility that the 100-nucleotide pair periodicity might appear only in rather special conditions of digestion and possibly be due to a minor activity present in the commercial enzyme preparation used, we tested a wide range of incubation conditions. The intensity of the bands relative to the background turned out to be highest in the low ionic strength conditions used. The bands were also detectable, however, when nuclei were digested in the presence of up to 5 mM EDTA or 5 mM CaCl₂ or up to 150 mM NaCl. Concentrations of CaCl₂ or EDTA in excess of 10 mM prevented degradation altogether. Variation of the pH between 4.0 and 7.5, and a change to phosphate and acetate buffers, did not abolish the pattern. It was found reproducibly with several batches of DNase II as well as with a preparation of DNase II prepared in the laboratory of G. Bernardi¹². The pattern is also not an exclusive property of mouse nuclei since it was found with rat liver^{7,8} as well as calf thymus nuclei (M. Steinmetz, R. E. Streeck and H.G.Z., manuscript in preparation).

It is impossible to differentiate quantitatively between DNA falling into the 100-nucleotide pair repeat pattern and randomly cleaved DNA which contributes to the background in the gels. Therefore we cannot rule out the possibility that only a certain part of the nuclear material is cleaved with a 100-nucleotide pair periodicity in these experiments.

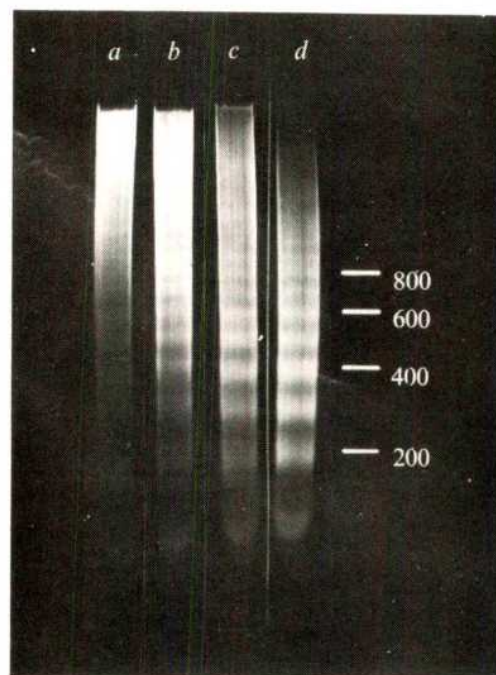


Fig. 2 Kinetics of digestion of mouse liver nuclei with DNase II. Nuclei were digested with DNase II (120 U ml⁻¹) as described in Fig. 1. Samples containing 5 μ g of DNA were withdrawn after 5 (a), 8 (b), 11 (c) and 18 (d) min, and analysed on a 1.8% Agarose gel. The molecular weight scale (in nucleotide pairs) was derived from *Eco*RII-digested mouse satellite DNA (Fig. 1).

Protease activity, as one potential source of artefact, was monitored during DNase II action by analysis of the histone patterns. In the presence of PMSF, which was always used, no differences were observed between unincubated samples and samples incubated without enzyme, with DNase II, and with micrococcal nuclease for the incubation periods used.

DNase II resembles DNase I in its ability to introduce single-strand nicks at 10-nucleotide intervals into the DNA. In denaturing 6% polyacrylamide gels¹⁶, DNA from DNase II-digested nuclei gave a series of strong bands with a 10-nucleotide repeat up to a fragment of 180 nucleotides, similar to the pattern found with DNase I⁵. In addition, broader bands were present at 200, 300, 400, 500 and 600 nucleotides.

New chromatin subunit?

Nucleosomes can be isolated as deoxyribonucleoprotein particles by sucrose gradient centrifugation. It was conceivable, therefore, that by this technique discrete deoxyribonucleoprotein particles containing 100-, 200-, 300-, and so on, nucleotide pair DNA, could be observed after digestion with DNase II. Figure 3 shows that this is not the case in the conditions used, but that instead particles are seen which correspond in their sedimentation properties to those produced with micrococcal nuclease.

Quantitatively, however, the patterns do differ. In the DNase II digests, there is always, even after extensive digestion, little material in the monomer region and the peaks with even numbers are more pronounced than the others. If the DNA from the gradient fractions is analysed in Agarose gels (Fig. 3B) it is evident that this DNA is of the 100-nucleotide pair repeat type, but the rapidly sedimenting material contains not just the high multiples of the repeat unit but the entire spectrum of bands starting with the 100-nucleotide pair fragment. We interpret this to mean that one site of attack for DNase II is between nucleosomes, and that cleavage at the second site, within the nucleosome, does not lead to the appearance of a smaller particle, but that instead the integrity of the subunit is maintained. In addition, the small amount of monomeric nucleosomes and the high proportion of dimers and tetramers in the sucrose gradients indicate that these nucleosomes have a high tendency to remain associated even when the internucleosomal DNA is broken.

Histone H1 is implicated in internucleosomal interaction and might be responsible for aggregation between nucleosomes. To test this, H1 was dissociated by 0.6 M NaCl after DNase II digestion. This changed the sucrose gradient profile drastically (Fig. 4A). Much more monomer was produced at the expense of higher multiples, and the DNA analyses of the gradient fractions indicate that the aggre-

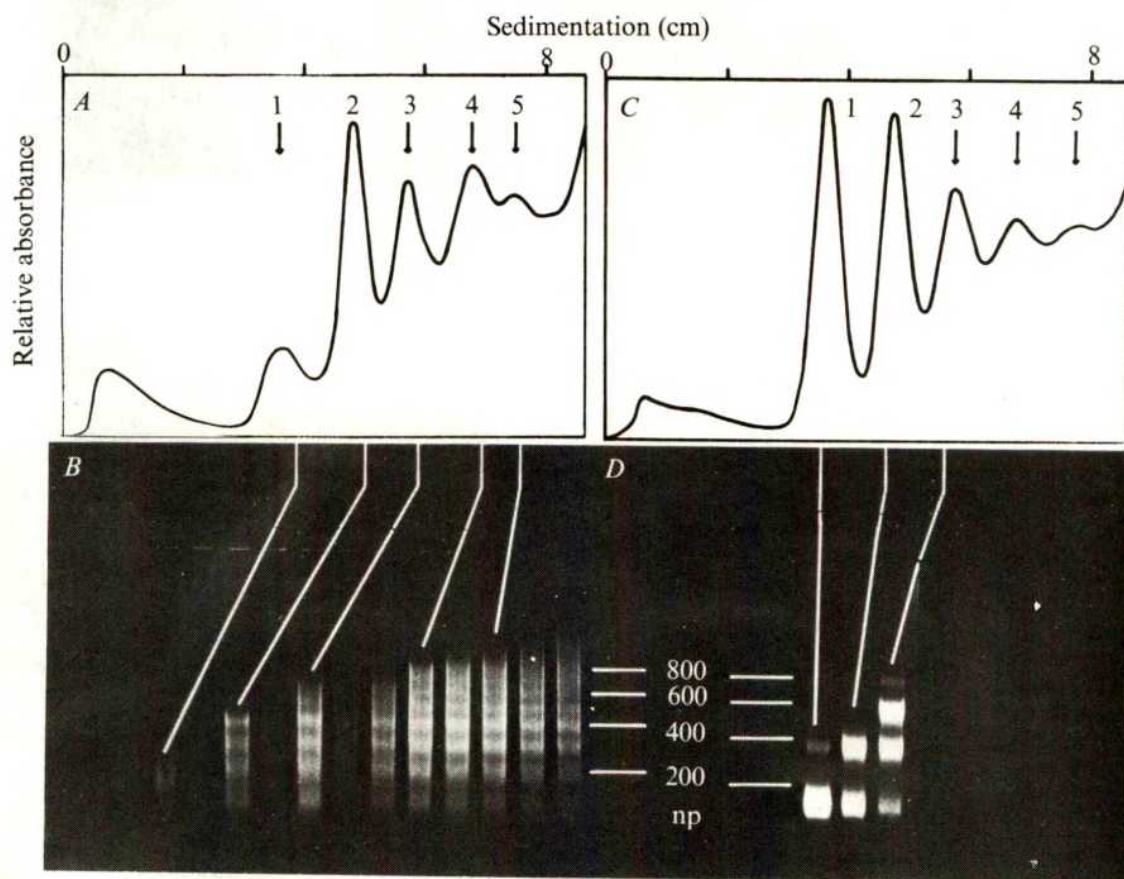


Fig. 3 Sucrose gradient fractionation of nuclease digests of mouse liver nuclei. Nuclei were isolated as in Fig. 1, except that 0.2% Triton X-100 and 1 mM PMSF were included in the homogenisation medium. They were digested with DNase II (120 U ml^{-1}) for 60 min at 37°C in 10 mM Tris-HCl, pH 7.0, 1 mM PMSF or with micrococcal nuclease (10 U ml^{-1}) in identical conditions except that 1 mM CaCl_2 was present during incubation. At the end of the incubation 2 mM EDTA was added, and the mixtures were centrifuged at low speed ($4,000g$, 10 min). The pellet was resuspended in 0.2 mM EDTA, pH 7.0, insoluble material was removed by a low speed spin and the supernatant was applied to isokinetic sucrose gradients containing 1 mM EDTA, pH 7.0 ($C = 5\%$, particle density 1.51, ref. 17). This supernatant contained negligible residual DNase II activity as shown by control incubations of the supernatant at 4°C for 24 h. Centrifugation was in a Spinco rotor SW 41 for 13 h at 38,000 r.p.m. at 4°C . The gradients were fractionated from bottom to top as described¹⁷ and absorbance at 260 nm was monitored in a flow-through cell with a Zeiss spectrophotometer. A, DNase II digest; C, micrococcal DNase digest. The numbers refer to monomeric (1), dimeric (2) and so on nucleosomes. DNA was isolated from the nucleosome fractions of both gradients and analysed on 1.8% Agarose gels (B and D) as described in Fig. 1. The solid lines correlate the nucleosome peak fractions with the corresponding DNA samples. The molecular weight scale (in nucleotide pairs) was derived from *EcoRII*-digested mouse satellite DNA (Fig. 1).

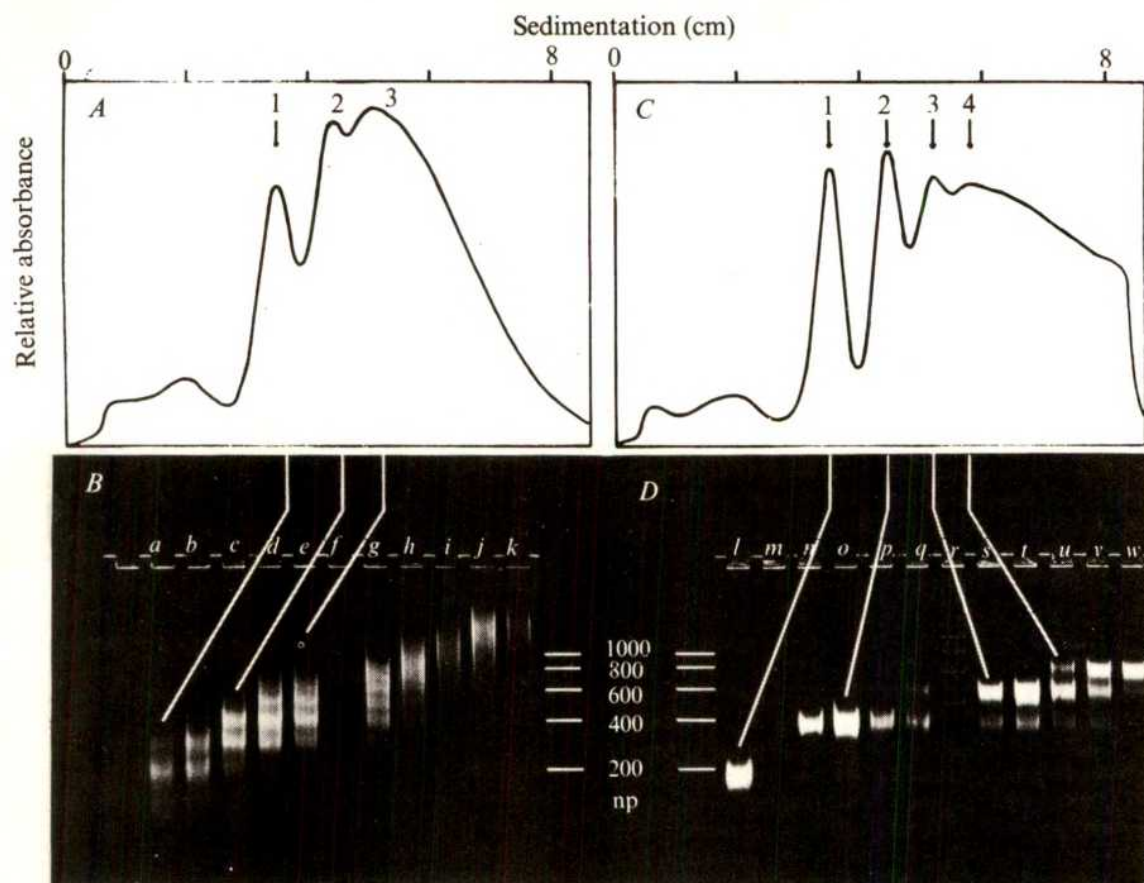


Fig. 4 Sucrose gradient analyses of nuclease digests after exposure to 0.6 M NaCl. The same nucleosome-containing supernatants as those from Fig. 3 were made 0.6 M in NaCl and then analysed as described in Fig. 3 without inclusion of NaCl in the gradients. *A* and *B*, DNase II; *C* and *D*, micrococcal nuclease digests. Slots (*f*) and (*r*) contain *Eco*RII-digested mouse satellite DNA.

gation between nucleosomes was strongly reduced (Fig. 4*B*). Nevertheless, the integrity of the nucleosome was maintained in spite of the internal cut. There is also aggregation between nucleosomes in micrococcal nuclease digests which

is abolished by exposure to 0.6 M NaCl. This is not so apparent from the sucrose gradient profiles (Figs 3*C* and 4*C*), but clearly seen in the gel analyses of sucrose gradient fractions (Figs 3*D* and 4*D*). In both micrococcal nuclease and DNase II digests nucleosomes sediment more slowly after exposure to 0.6 M NaCl, indicative of the loss of histone H1 and possibly a conformational change in the nucleosomal arrangement produced by the salt step.

DNase II digestion patterns sensitive to ionic environment of chromatin

For some experiments chromatin was prepared from the nuclei as a first step towards a more defined system. Mouse liver nuclei were suspended several times in 20 mM EDTA, 80 mM NaCl, pH 6.3 (ref. 18), and then in the usual 10 mM Tris-HCl (pH 7.0) and 1 mM PMSF. The unsheared gel-like material gave the typical pattern after digestion with micrococcal nuclease (Fig. 5*b*). The pattern obtained with DNase II from this chromatin depended very much on the ionic conditions. In the standard buffer, the DNA was cleaved by DNase II into a 200-nucleotide pair repeat pattern (Fig. 5*c*) similar to the micrococcal nuclease pattern. The 100-nucleotide pair repeat pattern

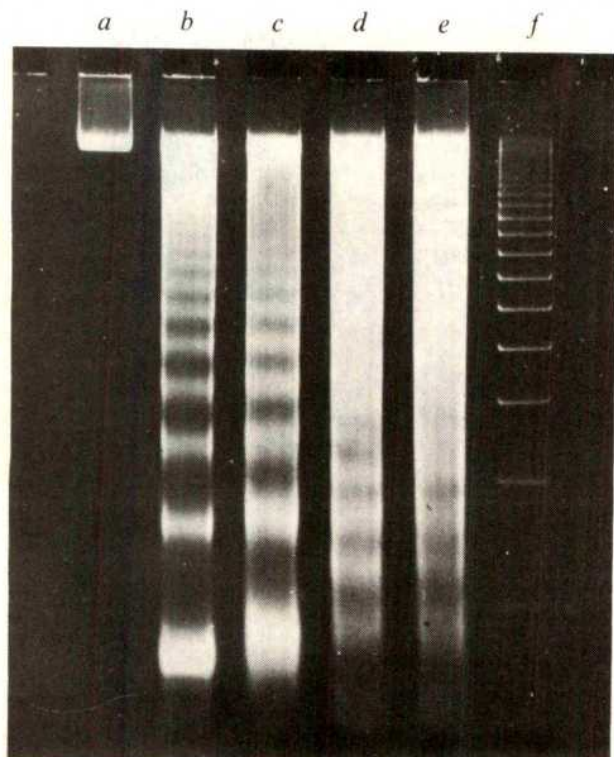
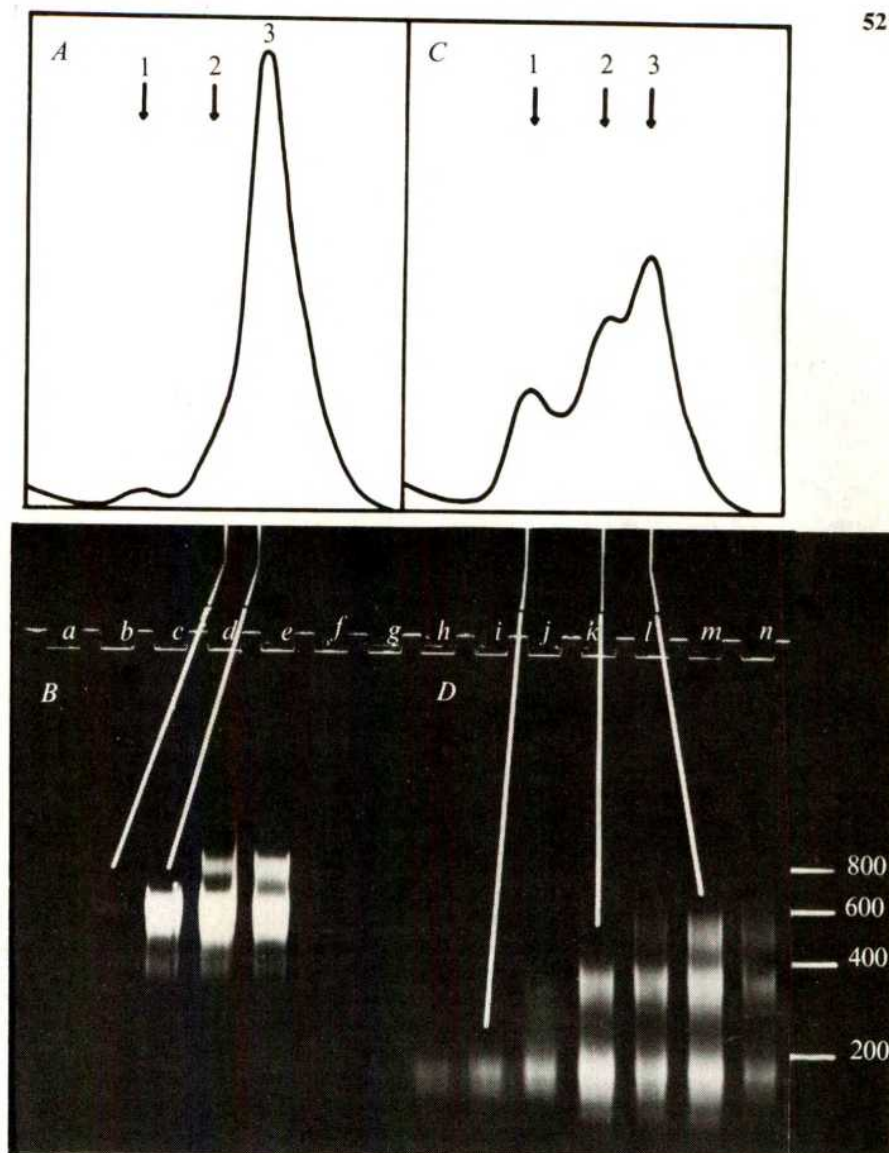


Fig. 5 Digestion of mouse liver chromatin with DNase II and micrococcal nuclease. Chromatin was prepared by extracting mouse liver nuclei three times with 80 mM NaCl, 20 mM EDTA, pH 6.3 (ref. 18). The resulting chromatin was washed twice in 10 mM Tris-HCl, pH 7.0 and digested in the same buffer in the presence of 1 mM PMSF for 40 min at 37 °C. Additions were: *a*, 1 mM CaCl_2 ; *b*, 1 mM CaCl_2 , micrococcal nuclease (25 U ml^{-1}); *c*, DNase II (500 U ml^{-1}); *d*, 1 mM CaCl_2 , DNase II (500 U ml^{-1}); *e*, 150 mM NaCl, DNase II (500 U ml^{-1}). Slot (*f*) contains an *Eco*RII digest of mouse satellite DNA (Fig. 1).

Fig. 6 DNase II digestion of a trimeric nucleosome fraction from a micrococcal digest. The micrococcal digest of nuclei was prepared and fractionated on sucrose gradients as described in Fig. 3. The material corresponding to trimeric nucleosomes was collected, concentrated and adjusted to 0.2 mM EDTA, pH 7.0, by pressure dialysis (Amicon), recentrifuged in a sucrose gradient as before and concentrated again by pressure dialysis. It was then incubated in 10 mM Tris-HCl, pH 7.0, 0.2 mM EDTA and 1 mM PMSF for 5 min without (*A* and *B*) and with DNase II (90 U ml⁻¹) (*C* and *D*). The incubation mixtures were analysed in sucrose gradients containing 1 mM EDTA, pH 7.0, in a Spinco rotor SW 56 for 14 h at 32,000 r.p.m. at 4°C. In (*A*) and (*C*) the gradient profiles are shown, and in (*B*) and (*D*) analyses of the DNA from the gradient fractions as correlated by the solid lines.



appeared, however, if DNase II digestion was carried out after prior addition of divalent or monovalent cations (Fig. 5*d* and *e*). This shift from a 200-nucleotide pair to a 100-nucleotide pair pattern coincides with strong contraction of chromatin known to be caused by these salt conditions¹⁰.

200-Nucleotide pair repeat pattern with DNase II

Cleavage at 200-nucleotide pair intervals by DNase II at low salt conditions might take place at either the inter- or the intranucleosomal sites. To decide between the two possibilities, sucrose gradient analyses and codigestion experiments were carried out with micrococcal nuclease.

Sucrose gradients of DNase II digests, which on deproteinisation yielded the 200-nucleotide pair repeat pattern, gave the standard nucleosome pattern (not shown), as would have been expected only if cleavage had occurred between nucleosomes. To define this region further relative to the region where micrococcal nuclease cleaves, trimeric nucleosomes were isolated by sucrose gradient centrifugation from a micrococcal nuclease digest of mouse liver nuclei and further digested with DNase II. In standard conditions, without any salt added, dimeric and monomeric nucleosomes were produced, containing only DNA fragments of the 200-nucleotide pair repeat length (Fig. 6). This proves that the regions cleaved by micrococcal nuclease and by DNase II in these conditions must overlap either completely or at least partially.

To find out whether DNase II digestion of trimeric nucleosomes also depends on the ionic conditions, digestion was carried out in the presence of CaCl₂ or MgCl₂ at concentrations between 0.5 and 2 mM (ref. 8). The patterns were clearly different when divalent cations were present: there were DNA bands in the gel at 200 and 400 nucleotide pairs and there was also DNA migrating in between these bands with indications of bands around 300 and 500 nucleotide pairs. The patterns were not sufficiently defined, however, to identify them clearly as of the 100-nucleotide pair repeat type.

Analogous results were obtained with two other nucleosome fractions, one containing tetramers, the other pentamers to dodecamers. Clear 200-nucleotide pair repeat patterns were seen after digestion with DNase II in standard conditions and a high background and indications of intermediate bands when divalent cations were present in the incubations.

DNase II and structural features of the nucleosome

The major difference between DNase II and other nucleases is the preferential double-strand cleavage at one site within the nucleosome. This difference might be due in part to the ability of DNase II to introduce both double-strand and single-strand cuts with high frequency¹².

It is conceivable that double-strand cleavage in the nucleosome occurs more easily after several primary single-strand cuts. At one site, cleavage would then occur in both strands. The stronger propensity of DNase II to nick DNA intranucleosomally might also be responsible for the high background of DNA in the digestion patterns. This interpretation is supported by the finding in our laboratory that DNase I, a nuclease which introduces predominantly single-strand cuts into DNA, also produces a 100-nucleotide pair repeat pattern, albeit much weaker and with an even higher background of DNA between bands⁸. That DNase I has a preferred site of attack within the nucleosome has been proposed on the basis of a strong subnucleosomal band in denaturing polyacrylamide gels²⁰.

What features of the chromatin structure lead to DNase II cleavage at the intranucleosomal site? Splitting at this site is symmetrical as far as the DNA is concerned, and in view of the symmetrical construction of the nucleosome from two of each of the core histones, it is tempting to think that DNase II might split at a site of symmetry within the nucleosome. Several models for the nucleosome structure incorporate such a symmetry element (for example refs 21 and 22). Extending the symmetry to include histone H1 would require two molecules of H1 per nucleosome. This would be appealing, but is not essential for the concept of DNase II cleavage at a site of symmetry.

DNase II as a probe into conformational states of chromatin

In chromatin, the accessibility of the intranucleosomal cleavage site to DNase II correlates with a reversible transition from an extended to a condensed conformational state. In addition to the ionic environment, temperature has been found to affect the conformational state: splitting at the second site is virtually absent at 0 °C in nuclei but the accessibility increases with temperature until, at 37 °C, both sites are cut equally well^{7,8}. In the presence of divalent cations, the temperature effects are not observed⁷. H1 is known to be involved in salt-induced aggregation of chromatin. H1-depleted calf thymus chromatin appears to have lost the intranucleosomal cleavage site, and this suggests that H1 is involved in the shift from the 200-nucleotide pair to the 100-nucleotide pair repeat pattern

(M. Steinmetz, R. E. Streeck and H.G.Z., manuscript in preparation).

Isolated oligomeric nucleosomes still have the ability to respond to ionic changes in DNase II digestion. They should therefore provide a system in which to study the parameters involved in this response. Detailed information on the effect of size and concentration of the isolated nucleosomes might tell to what extent aggregation or internal structural rearrangements of the nucleosome play a role. The results obtained with chromatin likewise do not enable us yet to decide if the opening and closing of intranucleosomal cleavage sites is due to conformational changes within the individual nucleosome or if it is the result of defined interactions between nucleosomal arrays, perhaps leading to a higher order packing of the chromatin fibril. It is clear, however, that nuclease digestion experiments can yield information not only on basic structural aspects but also on conformational states of nucleoproteins possibly related to higher structural orders.

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Thermal denaturation profiles and the structure of chromatin

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Analysis of the melting profile of DNA stripped of non-histone proteins and histone H1 allows definite structural conclusions to be drawn; conversely, it is possible to calculate what a particular chromatin model's melting curve will look like.

shown here that the shape of the melting profile can be calculated for a given model of the chromatin structure, and conversely that explicit structural conclusions can be drawn from the observed melting curves.

Theoretical considerations

The theoretical analysis makes use of the statistical mechanics of helix-coil transitions of two-stranded polynucleotides⁷. Neglecting the generally small effect of local heterogeneity of composition, one may divide the DNA helices into four classes, in respect of melting behaviour. These are illustrated in Fig. 1a and comprise (1) helix with free ends (*o,o*-helix), (2) helix joined at both ends to more stable helices (*h,h*-helix),

OPTICAL melting profiles of intact and partially stripped chromatin have been studied in many laboratories¹⁻⁵, and have been resolved into separate transitions, associated⁶ with free DNA and the DNA helices complexed with protein. It is

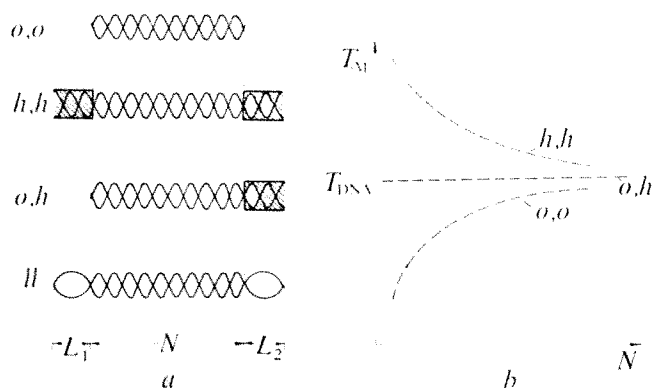


Fig. 1 *a*, Thermodynamically distinguished helix types: *o,o*, *h,h* and *l,l*-helices. *b*, Relationship between melting temperature T_m and helix length N , for helices of *o,o*-, *h,h*- and *o,h*-type respectively.

(3) helix with one end free and the other joined to a more stable helix (*o,h*-helix), (4) helix between two loops, consisting of less stable, melted helices (*l,l*-helix). We now consider how the melting temperatures (T_m) of such helices depend on their lengths. Helices of the *o,o*-type have been fully treated^{8,9}; the T_m falls in a known manner with decreasing length. For an *h,h*-helix, on the other hand¹⁰, T_m rises with decrease in helix length. The other two types of helix have not previously been treated. The statistical mechanical analysis is given later.

The results are as follows: the melting of an *o,h*-helix is independent of its length; for an *l,l*-helix the situation is more complex; the relation between T_m and chain length depends on the size of the nucleotide loops at the ends. In the limit of zero loop length the configuration becomes equivalent to an *h,h*-helix, and as the loop grows, the T_m drops. It follows from the partition function (see statistics) that the T_m of an *l,l*-helix may be higher or lower than that of an infinite helix, but is always higher than that of an *o,o*-helix of the same length. The laws governing the melting of different types of helix are shown in Fig. 1*b*. A precise quantitative analysis is limited by lack of information about the magnitude of the helix initiation constant, σ , at low ionic strengths. At this stage therefore, only a semi-quantitative interpretation of the melting profiles can be attempted.

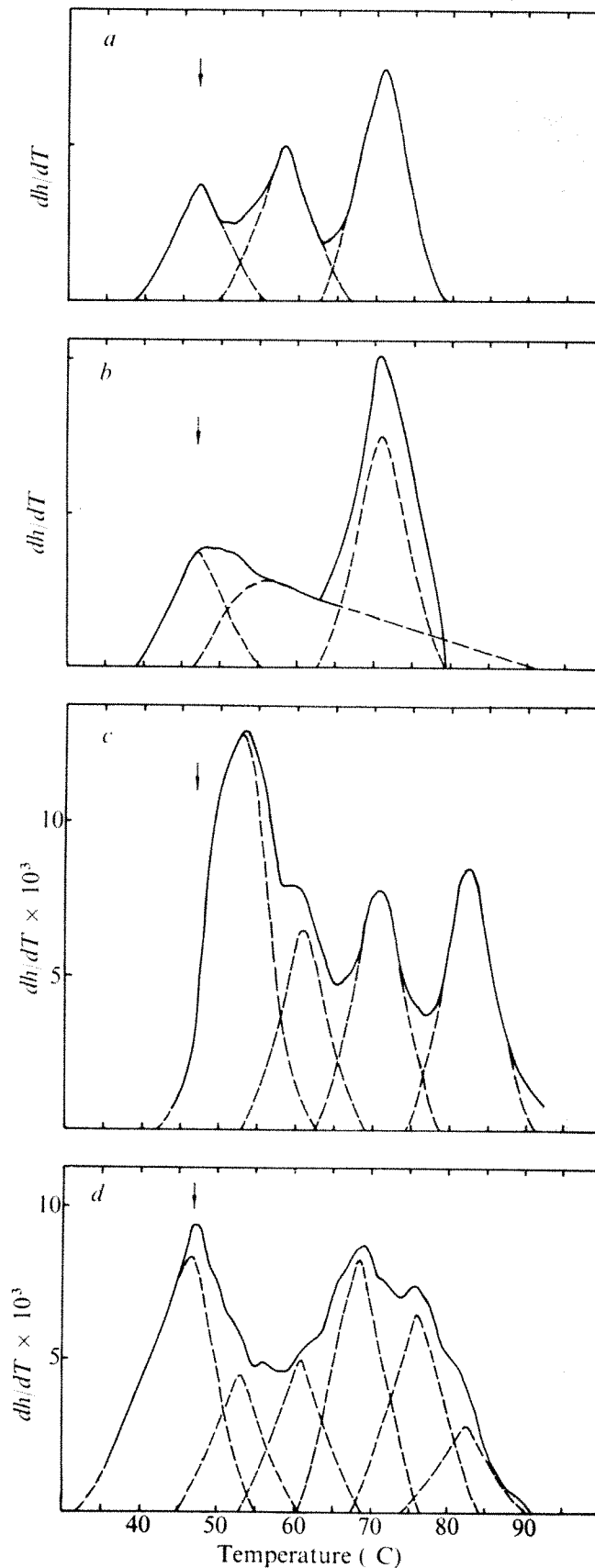
Chromatin models

The current view of chromatin structure¹¹⁻¹⁵ is based on a repeating unit of about 200 base pairs, 140 of these being in the form of a complex with protein (nucleosome), and the remaining 60 of a spacer of free DNA. On treatment with 0.6 M NaCl, the non-histone proteins and histone H1 are lost, but the periodic structure remains. In this material, called DNP 0.6, the nucleosomes contain only DNA and eight histone molecules, most probably two copies of each remaining species¹⁶⁻¹⁸.

We now consider the predicted melting behaviour of two possible models for DNP 0.6.

- **Equally Spaced Nucleosomes.** If the nucleosomes are all identical and spaced at equal intervals along the DNA, a melting profile encompassing three transitions is to be expected: that of naked DNA at the ends of the molecule (*o,h*-helices) with T_m as for free DNA (T_m^0); that of free DNA between nucleosomes (*h,h*-helices), with (as noted by Subirana³) $T_m > T_m^0$; and that of the nucleoprotein complex, which is associated with a higher numerical enthalpy of transition, both because of electrostatic stabilisation of the charged structure and the nature of the helix (*l,l*). The corresponding T_m will be higher than that of simple nucleosomes, in which the DNA has free ends. If the T_m of the second transition is sufficiently high, the second and third transitions may be brought into coincidence.

Fig. 2 Schematic derivative melting profile for a DNA duplex: *a*, with nucleosomes equally spaced along its length; and *b*, with randomly distributed nucleosomes; *c*, experimental profiles for DNP 0.6; and *d*, for the same material after sonication for 8 min at 1.2 A in an MSE sonicator. The DNP 0.6 was obtained from salt-extracted chromatin²⁰ after treatment with 0.6 M NaCl and chromatography on a Sepharose 4B column, 40×4 cm. Thermal denaturation profiles were measured in 0.25 mM Na EDTA, pH 8.0, with an instrument previously described^{21,22}. Differentiation was performed graphically, for intervals of 4 °C. The arrow corresponds to T_m for free DNA.



The breadth of the transitions will in the first place reflect the compositional heterogeneity of the duplex. The first and second transitions will in addition be broadened because of the finite lengths of the cooperative units, whereas the third phase (140 base-pair helix) should not be subject to broadening, and will resemble in shape the melting of free DNA. The areas under the peaks in the derivative melting curves will reflect the relative concentrations of the three types of structure. The predicted melting profile of this model for DNP 0.6 is shown in Fig. 2a.

• Randomly Spaced Nucleosomes. If alternatively the nucleosomes are randomly distributed along the DNA, the transition profile will be different (Fig. 2b). The second transition will be replaced by a continuous spread of melting of naked DNA. Segments short enough to have an intrinsic T_m greater than that of the nucleosomes will melt with the latter, since this implies a switch from h,h to l,l character, and the area under the corresponding peak in the derivative melting profile will be greater than would correspond to the DNA content of the nucleosomes. The third peak will be broadened by the distribution of loop lengths.

Analysis of melting profiles

The melting profile for DNP 0.6 is shown in Fig. 2c. There are four peaks, the three highest ($T_m = 61, 72$ and 82°C) corresponding to the most prominent components in whole chromatin, whereas the lowest ($T_m = 56^\circ\text{C}$) has no counterpart in chromatin. The areas under the peaks corresponding to the two highest temperature transitions correspond to the total protein content (see also ref. 3). Thus the first two transitions represent the melting of free DNA, and the other two that of complexed DNA. The first transition is broadened relative to that of free extracted DNA. The fourth is unchanged, and the second and third also do not seem to be significantly broader.

In analysing these curves we note first that there is no component of $T_m = T_m^0$, so that the amount of terminal naked

DNA is negligible. The presence of two peaks of melting of naked DNA with $T > T_m^0$ implies two populations of spacers (h,h -helices) between nucleosomes. The first and second transitions correspond to long and short segments of DNA helix respectively. The two transitions of protein-DNA complexes are sharp, and evidently represent standard, effectively homogeneous, populations containing relatively long segments of DNA with nucleotide distribution not significantly different from that of the whole DNA. Three possible reasons for the existence of these two latter (l,l -helix) transitions may be envisaged: (1) the presence of two regions differing in thermal stability within each nucleosome, (2) the existence of both long and short spacer regions between the nucleosomes; the latter would melt at temperatures determined by the length of the spacers which define the loop sizes, (3) the existence of two structurally distinct types of nucleosome.

We have found (to be published) that the relative areas under the two peaks in question depend strongly on the ionic strength, the sum of the two remaining constant.

This observation renders the first explanation improbable. It has been reported¹⁹ that mononucleosomes prepared by limited enzyme digestion melt in a single phase with a $T_m = 76^\circ\text{C}$; this is between the T_m values of the two l,l transitions. Since a l,l -helix necessarily melts at a higher temperature than the same helix in a o,o situation, the material melting in the 72°C peak cannot represent the same type of structure as that melting in the 82°C peak if both are normal nucleosomes. We therefore rule out the second explanation, to be left with the third.

The analytical procedures described above are strengthened by data obtained with ultrasonically fragmented material (Fig. 2d). The following features stand out:

• A new peak with $T_m = T_m^0$ (47°C) appears, which accounts for the melting of more than half the naked DNA. Thus the sonic fragments are short, consisting mainly of one to three nucleosomes.

Statistical mechanical analysis

The partition function Z for the ensemble of partially melted configurations of a double helix of N base pairs, in general depends on s the equilibrium constant for the addition of a base pair to the end of a helical region and σ the nucleation constant. In terms of the zipper model, whereby we ignore configurations with more than one helical region, we may write

$$Z(N) = \sum_{n=0}^N s^n w_n \quad (1)$$

where w_n is the statistical weight of configurations with n base pairs. The fraction of possible base pairs that are intact may then be written

$$f = \frac{1}{N} \frac{d \ln Z}{d \ln s} = \frac{\sum_{n=0}^N n s^n w_n}{\sum_{n=0}^N s^n w_n} \quad (2)$$

For helices in the o,h situation, w_n is unity for all N , since we assume that melting proceeds from one end of the helix only.

At the melting temperatures (T_m), $F_{o,h} = \frac{1}{2}$, and it may be shown that this occurs at $s = 1$; thus for o,h helices T_m is independent of N .

For helices in the l,l situation there are $N-n+1$ ways in which the helix of n base pairs may be situated between the two loops. We assume that the two loops contain L_1 and L_2 nucleotides and write $L_1 = 2(N_1+1)$ and $L_2 = 2(N_2+1)$.

Each of the $N-n+1$ configurations must be weighted according to the probability of ring closure. An approximation⁶ gives L^{-c} , where c is a constant, as the appropriate weight for a loop of L nucleotides. It then follows that

$$w_n = \sigma \sum_{k=1}^{N-n+1} \left[4(N_1 + N - n + 2 - K)(N_2 - K) \right]^{-c} \\ \text{for } 1 \leq n \leq N - n + 1 \\ = 2^{-c}(N_1 + N_2 - N + 1)^{-c} \text{ for } n = 0$$

This expression may be substituted into equation (2) to yield $f_{l,l}$, which is clearly dependent on N , N_1 , N_2 , s and σ .

In the event that $L_1 = L_2 = 0$, however, the situation approximates to that of a h,h -helix (melting from both ends), and in the limit of very large L_1 and L_2 it approximates to the o,o situation. We therefore suppose that the plot of T_m versus N for a l,l -helix with finite loops would lie between the curves for h,h and o,o helices, at a position depending on the size of the loops. Consequently at constant N , T_m decreases with increasing size of the loops. It then follows that the T_m of finite helices in the l,l situation can be higher or lower than that of an infinite helix, but is always higher than the T_m of a helix of the same length but in the o,o situation.

• Concomitantly the other peaks associated with naked DNA decrease, showing that the intact DNP 0.6 contained no short segments of naked DNA, for otherwise sonication would have brought about an increase in the low-melting peaks.

• The peak of $T_m = 72^\circ\text{C}$ is shifted to lower temperatures by about 2.5°C , while the peak of $T_m = 82^\circ\text{C}$ is diminished by two-thirds in amplitude. At the same time a new peak appears at 76°C . The latter presumably corresponds to mononucleosomes²⁰. The transition at 82°C arises from the melting of nucleosomes in long fragments (*I,I*-helices), and that at 76°C from nucleosomes with free ends (*o,o*-helices). The peak at 72°C cannot be identified with a nucleosome in any state. It is present in DNP 0.6 but not in enzymically derived mononucleosomes. It may represent an altered or denatured nucleoprotein structure, which is not resistant to enzymic attack.

It is hoped to extend the quantitative scope of the analysis by determining the helix initiation constant, σ , at low ionic strength, which would allow a rather precise analysis of the lengths of the naked DNA segments in chromatin, and the evaluation of the thermodynamic characteristics of the nucleoprotein complexes.

The analyses of the melting profiles of DNP 0.6 allow the following conclusions to be drawn with considerable confidence: (1) DNP 0.6 consists of a long DNA molecule containing DNA-protein complexes separated by regions of naked DNA. (2) The melting temperatures of both naked DNA segments and DNA-protein complexes are not governed solely by their intrinsic thermodynamic characteristics (mean enthalpy and entropy), but depend strongly and in an interpretable manner on their lengths and positions in the structure. Earlier simplistic

interpretations of multi-phase transitions can no longer be sustained. (3) The melting transitions of naked DNA and of protein complexes (first two and second two peaks respectively) are completely separate. (4) The distribution of the nucleosomes along the DNA is independent of local nucleotide composition. (5) The material contains two different populations, each effectively homogeneous, of DNA-protein complexes, differing from each other in thermal stability and the mean lengths of the associated DNA.

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Electrified black holes

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Black holes with accretion disks are self-excited dynamos that generate large electric and magnetic fields. Along the spin axis they emit oppositely-directed narrow beams of high energy positrons and photons. The beams emanating from superholes are sufficiently powerful to explain extragalactic radio sources.

A BLACK hole has mass M , angular momentum J , and electrical charge Q , satisfying the relation

$$G^2 M^4 \geq J^2 c^2 - GM^2 Q^2 \quad (1)$$

where G is the gravitational constant and c is the speed of light. The equality sign denotes the maximum angular momentum and charge of the extreme Kerr-Newman metric. A spinning charged black hole has a magnetic dipole moment¹

$$\mathcal{M} = JQ/Mc \quad (2)$$

and at distances large compared with the gravitational length $R = GM/c^2$ the magnetic intensity obeys the familiar r^{-3} law. Normally, a large electric charge in a black hole would be rapidly neutralised by inflow of oppositely charged particles, and it has seemed reasonable therefore to suppose that charged black holes are not of great astrophysical importance. I shall attempt to show that a significant charge can in fact accumulate in black holes in quite general conditions, and that black-hole

electrodynamics may supply the long-sought-for explanation of the extended² and compact radio sources³.

Maximum electric charge

The maximum magnitudes of electromagnetic quantities, attainable in principle but not necessarily in practice, are denoted with a dagger. Thus a charge that contributes substantially to the mass of a black hole is approximately $Q_+ = G^{1/2} M$, and hence

$$Q_+/M \sim 10^{-18} e/m_p \quad (3)$$

where e/m_p is the charge-to-mass ratio of the proton. The corresponding electric potential is

$$\phi_+ \sim c^2/G^{1/2} \sim 10^{27} \text{ V} \quad (4)$$

and the electric field in the vicinity of a black hole is

$$E_+ \sim c^4/G^{3/2} \sim 10^{22} (M_\odot/M) \text{ V cm}^{-1} \quad (5)$$

(For $E_+ > 10^{16} \text{ V cm}^{-1}$, or $M < 10^6 M_\odot$, this field will break down spontaneously by the pair production of electrons.) The angular momentum that also contributes substantially to the mass is approximately $J_+ = GM^2/c$ and so the magnetic dipole moment is $G^{3/2} M^2/c$. In this case, the magnetic field intensity in the vicinity of the black hole is therefore

$$B_+ = E_+ \sim 10^{20} (M_\odot/M) \text{ G} \quad (6)$$

It is thus evident that black holes, in principle, can have the most intense macroscopic electromagnetic fields realisable in nature.

Gravity-induced electric charge

Eddington* (see also refs 5 and 6) showed that stars are positively charged, and that electric fields play an essential role in their internal structure. Within a star—such as the Sun—proton and electron gases contribute equally to the pressure gradient, whereas gravity acts mainly on the relatively massive protons; an electric field therefore mediates between the proton and electron gases. In effect, electrons have velocities greatly exceeding the escape velocity, and some therefore escape leaving the star positively charged with an internal electric field that retains the remaining electrons.

Let ψ and ϕ be the gravitational and electric potentials. In a star like the Sun the forces acting on a proton and an electron are approximately equal

$$-m_p \nabla \psi - e \nabla \phi = -m \nabla \psi + e \nabla \phi$$

and hence, neglecting the electron mass, m , the electric field is

$$-\nabla \phi = \alpha (m_p/e) \nabla \psi \quad (7)$$

where $\alpha = 0.5$. In more massive stars α approaches unity owing to the pressure of radiation acting on the electrons. From the divergence of equation (7) it follows that the charge to mass ratio of a star is

$$Q_*/M = G\alpha m_p/e \sim 10^{-36} e/m_p \quad (8)$$

where the gravity-induced positive charge Q_* is ~ 100 C/solar mass. The potential difference between the surface and centre of the Sun is $\sim 1,000$ V.

All self-gravitating systems containing free electrons have gravity induced positive charges of the order indicated by equation (8). The induced charge cannot be neutralised by the accretion of electrons nor can it be neutralised by surrounding a star with a negatively charged sheath. This is because the gravitational and electric fields exert everywhere a total force that acts equally on electrons and protons, and consequently an external ionised medium remains unpolarised. When Q is less (more) than Q_* , the external medium is polarised and there is an outflow (inflow) of electrons until Q_* is restored and the external medium is depolarised.

Black holes that form by stellar collapse have initial charge to mass ratios of the order given by equation (8) and

$$Q_*/Q_+ \sim (Gm_p^2/e^2)^{1/2} \sim 10^{-18} \quad (9)$$

Their electric potential $\phi_* \sim Q_* c^2/GM$ is

$$\phi_* \sim m_p c^2/e \sim 10^9 \text{ V} \quad (10)$$

and is independent of mass. With angular momentum J_+ the magnetic field intensity is

$$B_* \sim 10^{-18} B_+ \quad (11)$$

or $\sim 10^3$ G in the vicinity of the black hole.

Superholes

Almost all matter in the Universe has too much angular momentum, either initially to form into black holes or subsequently to be accreted by black holes. Angular momentum must therefore be removed and, in the case of accretion, a viscous accretion disk develops in which gas spirals in toward the black hole and angular momentum is transported away⁷⁻¹⁰. A weakly charged black hole thus has angular momentum $J_+ \simeq GM/c$ and approaches the extreme Kerr metric¹¹. An accretion disk generally lies in the rotational equatorial plane, and its orbital angular velocity has the same direction as the spin angular

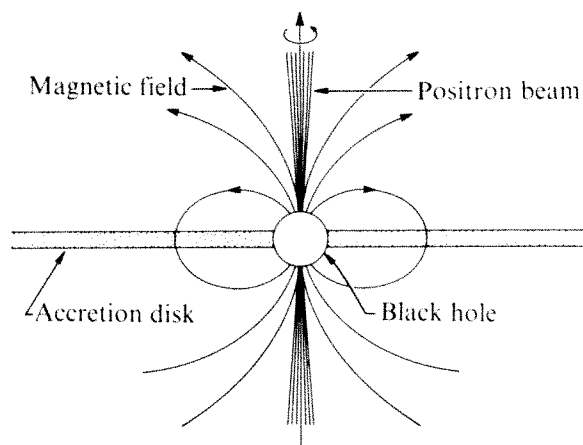


Fig. 1 A black hole with an accretion disk acts as a self-excited dynamo. The black hole rotates, it has an initial gravity-induced positive charge, and it generates a dipole magnetic field. The magnetic field induces a positive charge in the disk, and the charge accreted is therefore proportional to the charge already in the black hole. The charge in the black hole thus grows exponentially while its mass grows linearly with time. The production of high energy positron beams, that emanate from the polar regions, is discussed in the text.

velocity of the black hole. Black holes that form in matter-rich galactic nuclei accrete gas (also tidally disrupted stars) and become supermassive^{12,13}, attaining masses $\sim 10^8 M_\odot$ on a time scale $\sim 10^8$ yr. These supermassive objects are conceivably the primary sources of energy in quasars and in violent nuclear activity¹⁴.

A system consisting of a charged spinning black hole encircled by an electrically conducting accretion disk contains the basic elements of a self-excited homopolar generator. Such systems, if they actually exist, constitute the most powerful electrical machines in nature.

It is emphasised that throughout this treatment general relativistic subtleties are ignored, and all results are therefore only rude order-of-magnitude estimates.

Black-hole homopolar generator

A rotating accretion disk (see Fig. 1) of high electrical conductivity has an induced electric field

$$\mathbf{E} = -\mathbf{v} \times \mathbf{B}/c \quad (12)$$

where \mathbf{v} is the orbital velocity and \mathbf{B} is the axisymmetric magnetic field of the black hole.

The potential difference between the inner and outer regions of a disk in Keplerian differential rotation is

$$\phi \sim Qc^2/GM \quad (13)$$

and is of the same order as the Coulomb potential of the black hole. From the divergence of equation (12) the volume charge density in the disk is

$$\sigma = (\mathbf{v} \cdot \nabla \times \mathbf{B} - \mathbf{B} \cdot \nabla \times \mathbf{v})/4\pi c \quad (14)$$

Electromagnetic models in which trapped magnetic flux is accreted are considered elsewhere (R. V. E. Lovelace, unpublished; R. O. Blandford and R. L. Znajek, unpublished). The dragging inward of magnetic flux is ignored in this discussion and hence $\nabla \times \mathbf{B} \sim 0$. It follows, using cylindrical coordinates (r, ϕ, z) , that

$$\sigma = -(B_z/4\pi c) \partial(rv)/r \partial r \quad (15)$$

Since $v \propto r^{-1/2}$, and \mathbf{B} in the disk is antiparallel to $\nabla \times \mathbf{v}$ for a positively charged black hole, the charge density is therefore also positive.

The basic mechanism of the proposed generator is that positively charged matter is accreted by a positively charged black hole. The induced charge σ in the disk is proportional to the charge Q in the black hole and therefore Q grows faster than the mass M . (The surface charge on the disk is determined by boundary conditions, and is not accreted.)

Let \dot{M} be the rate that mass is accreted, and \dot{Q} the rate that charge is accreted; further, let ρ_R and σ_R be the mass and charge densities of the innermost stable region of the disk close to the event horizon; then

$$\dot{Q}/\sigma_R \sim \dot{M}/\rho_R \quad (16)$$

where

$$\sigma_R \sim Q(GM)^{3/2}/8\pi c^3 R^{7/2} \sim Qc^6/8\pi G^3 M^3 \quad (17)$$

from equation (15). The mass density ρ_R is proportional to M/R^2c , and therefore

$$\dot{M}/\rho_R \sim G^2 M^2/\mu c^3 \quad (18)$$

where μ is plausibly ~ 10 . Equation (16), with equations (17) and (18), now becomes

$$\dot{Q} \sim Qc^3/8\pi GM\mu \quad (19)$$

and according to this simplified treatment the black hole charge grows exponentially on a time scale

$$T \sim 8\pi GM\mu/c^3 \sim 10^{-3}(M/M_\odot) \text{ s} \quad (20)$$

The initial 'seed' charge is the gravity induced value Q_* , and hence

$$Q \sim Q_* \exp(t/T)$$

Radiative discharge

In many respects, electrified black holes are different from pulsars. The spin and magnetic dipole axes of a black hole are coincident, whereas pulsars are oblique dipole rotators¹⁵. Pulsars have magnetospheres maintained by particle emission from the surface of a neutron star, and currents intersecting the surface enforce the magnetosphere into corotation¹⁶. The event horizon of a black hole cannot emit particles (quantum mechanical emission¹⁷ is negligibly small for the masses considered) and is not intersected by closed current loops. A black hole, therefore, is not embedded in a pulsar-like magnetosphere, and the huge potentials generated are therefore not easily short-circuited.

Electrons are attracted and accelerate along magnetic field lines that converge on the polar regions of the black hole. Let r_D be the outer radius of the disk; then the field lines not intersecting the disk converge on the polar regions at angles

$$\theta \lesssim (R/r_D)^2 \quad (21)$$

to the spin axis. The Larmor orbit radii of infalling electrons are small compared with R , and for R/r_D in the range 10^{-2} to 10^{-3} the electrons are confined to field lines of $\theta \leq 2^\circ$ – 6° . (The accreted electrons streaming down these narrow cones, in spite of space-charge limitation, may prevent the black hole from attaining large electrical potentials. Preliminary estimates indicate that this is unlikely.)

When the potential of the black hole is large, the infalling electrons emit curvature radiation that initiates a pair-production discharge^{18–20}. The created electrons and positrons of energy γmc^2 also emit curvature radiation consisting of photons of energy $\sim \gamma^3 \hbar c/R_c$, where R_c is the radius of curvature of the magnetic field lines. These photons cut across field lines and produce further electron pairs. All electrons plunge into the black hole and the positrons accelerate away in a narrow beam. If the photon mean free path, λ , for pair production is greater than a scale height $\sim R$ there is no significant discharge and the potential continues to rise. When $\lambda < R$, however,

pair-production avalanches until the black hole is discharged to the point where $\lambda \sim R$. With $\lambda \sim R \sim R_c$, the steady-state potential is found to be

$$\phi_s \sim 3 \times 10^{10} (mc^2/e)(M/M_\odot)^{1/2} \quad (22)$$

from formulae used in pulsar theory (refs 19, 20 and I. Lerche, unpublished). Thus a black hole of $10^8 M_\odot$ attains a potential 10^{20} V. The rate of converting gravitational energy into high energy particles is $\sim Q\dot{Q}c^2/GM$, or $\sim \phi^2 c/8\pi\mu$ from equation (19), and according to equation (22) the energy output in the beams is

$$L \sim 10^{37} (M/M_\odot) \text{ erg s}^{-1} \quad (23)$$

Accreting black holes emit low energy photons, and an alternative cascade mechanism is therefore possible (R. O. Blandford and M. J. Rees, unpublished). An infalling electron of energy γmc^2 inverse-Compton scatters a low energy photon to energy $\sim \gamma^2 kT$, where T is the temperature of ambient blackbody radiation²¹. The high energy photon now pair produces through photon-photon interactions, and the process avalanches if the photon mean free path, λ , is less than a characteristic distance $\sim R$. When $\gamma \lesssim mc^2/kT$, then $\lambda \sim (n\sigma_T)^{-1}$, where n is the blackbody photon density and σ_T is the Thomson cross section. In this case the vacuum breaks down at $\phi \sim 10^{12} T_4/T$ V when

$$M/M_\odot \lesssim 10^8 T/T_4 \quad (24)$$

and $T_4 \equiv 10^4$ K. Thus $10^8 M_\odot$ may be a limiting mass for the production of high energy beams. This type of breakdown, however, is not self-regulating; for if $\gamma > mc^2/kT$, then $\lambda \propto \gamma$, and the discharge quenches until the potential rises to the point where curvature radiation breakdown occurs in which $\lambda \propto \gamma^{-3}$.

Radio sources

Strong radio sources^{2,3} have energy outputs typically in the range 10^{42} – 10^{45} erg s⁻¹; a conspicuous characteristic feature is their double structure consisting of lobes of radio brightness extending in opposite directions from a quasar to an optically bright galactic nucleus; the extended double sources have estimated separations 10^5 – 10^7 light yr, whereas compact double sources have separations 10 – 10^2 light yr; also extended and compact sources often share the same optically bright central region, as in Cygnus A, and the radio brightness centres of extended and compact regions tend to be colinear. Various authors^{22–25} have advocated 'twin-beam' models for explaining double radio sources and shown that such models, in which oppositely directed beams of waves and relativistic particles created by quasars and galactic nuclei and lasting for 10^8 – 10^9 yr, have many attractive features.

The possibility that black holes have oppositely directed narrow beams of high energy particles is therefore of immediate interest in the study of radio sources. If it is granted that supermassive black holes with accretion disks exist, then we are provided with a natural mechanism that is capable of producing beams of sufficient power to excite radio sources. Positron-photon beams with entrained electrons will travel to great distances and energise the external magnetised medium. Thus, according to equations (22) and (23), superholes of mass $10^8 M_\odot$ emit high energy beams ($\gamma \sim 10^{14}$) with a power output of $\sim 10^{45}$ erg s⁻¹.

The specific properties of radio sources that can be explained by beamed emanations from electrified superholes are:

(1) Double structure: the two-component basic structure, often possessing remarkable symmetry, is the result of identical and oppositely directed narrow beams. Asymmetries and 'U' shapes (as in the 'head-tail' sources NGC 1265 and 3C 129 in rich clusters) are plausibly the result of density variations and relative motions of the intergalactic medium.

(2) Directional memory: Cygnus A and 3C 236 have compact double components aligned in the same direction as their extended double components, and the directional memory in these and other sources is provided by the spin axis of the

electrified superhole. Slow precession of the spin axis on a time scale of millions of years, owing to dynamic interaction with mass distributed in the nucleus, may explain the 'S'-shaped sources.

(3) Brightness distribution: the high energy beams progressively become more intense as the superhole grows in mass. So, in accord with observations²⁶, less luminous (therefore younger) sources have bright inner regions excited by greater beam intensity, whereas more luminous (older) sources have bright outer regions excited by beams that have attained great intensity over long periods of time.

Detailed investigations of electrified black holes will undoubtedly disclose more complex behaviours, such as, for example, non-quiescent discharges and beam pulsations on time scales $\sim GM/c^3$ of minutes to hours. These rapid fluctuations would be smoothed out in the extended radio brightness regions, but if 'core-halo' sources are two-component sources seen end on, the fluctuations might be detectable in the radio emission from the central milli-arcsecond structure of the core.

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letters to nature

Search for parity non-conserving optical rotation in atomic bismuth

THE Weinberg-Salam^{1,2} theory of the electromagnetic and weak interactions has been successful in predicting the neutral current interactions observed in neutrino experiments. An important further test for the theory is the experimental observation of predicted³ parity non-conservation in atoms. We are carrying out two related though distinct experiments to search for parity non-conserving optical rotation in atomic bismuth close to the allowed M_1 transitions from the ground state, $\lambda = 648$ nm (at Oxford) and $\lambda = 876$ nm (at Washington). Although our experiments are continuing we feel that there is sufficient interest to justify an interim report.

The predicted optical rotation angle close to the M_1 transition $i \rightarrow f$ is given by

$$\phi_p = \frac{-4\pi(n-1)LR}{\lambda}$$

where n is the refractive index and λ is the wavelength.

$$R = \frac{\text{Im}\langle f|E_1|i\rangle}{\langle f|M_1|i\rangle}$$

is the ratio of the parity non-conserving E_1 matrix element to the normal M_1 one. ϕ_p therefore has a dispersive dependence on frequency close to resonance. At resonance there is, of course, also absorption which limits the useful optical depth, $(n-1)L$, which can be used. For one absorption length at line centre the optical rotation at the dispersion peaks is $\pm R/2$.

A number of relativistic central field calculations of R have been made⁴⁻⁷ using both semi-empirical and Hartree-Fock methods. The ± 25 per cent agreement in the results is very satisfactory considering the differing starting points. We take

as average central field values $R_{876\text{ nm}} = -3 \times 10$, $R_{648\text{ nm}} = -4 \times 10^{-7}$, for a Weinberg angle $^{1,2} \sin^2 \theta = 0.35$.

The basic method used in the two experiments is the same: a brief description of the Washington experiment appeared recently⁸. In each experiment laser light at a frequency close to the M_1 transition is passed through two crossed polarisers between which is the bismuth oven ($T \simeq 1,500$ K). The transmitted light intensity has the form

$$I = A(\phi_p + \phi_M + \phi_R)^2 + B$$

where A and B are constants, ϕ_p is the optical rotation angle defined above, ϕ_M is an additional modulated angle produced by a Faraday cell and ϕ_R is any residual angle caused, for example, by misalignment of the polarisers. The basic signal, I_M , is that part of I which reverses with ϕ_M , normalised against changes in laser intensity and bismuth transmission. It is proportional to $\phi_M(\phi_p + \phi_R)$. The contributions from ϕ_p and ϕ_R are distinguished by the dispersive form of the rapid frequency variation of ϕ_p close to resonance.

There are a number of important differences between the experiments: (1) the pulsed parametric laser required at 876 nm has a linewidth too large to allow resolution of the individual hyperfine components which are separately observed in the 648-nm experiment. (2) The 648-nm line is overlaid by a molecular spectrum which limits the usable optical density to about 1 atomic absorption length. This difficulty is absent at 876 nm where an appreciably higher optical path length can be used. (3) Because the linewidth is much narrower in the Oxford experiment, the Faraday rotation in the bismuth caused by background magnetic fields is larger. To avoid the difficulty the oven is magnetically shielded and the experiment is designed to discriminate against bismuth Faraday rotations. (4) Largely because of the differences (1) to (3), different procedures have been adopted for picking out the sharp frequency dependence of the ϕ_p dispersion curve. In the Washington experiment this is achieved by repeatedly sweeping

the laser frequency through the resonance region while the signal I_M is accumulated in a multichannel analyser. The result can be fitted to the appropriate dispersion shape for several bismuth optical path lengths to subtract out the ϕ_R background. In the Oxford experiment the laser frequency is switched between two points of equal bismuth Faraday rotation, but opposite optical rotation. A small residual frequency dependent component of ϕ_R is subtracted out by repeating the experiment with a reduced bismuth optical path length.

Our results are as follows:

$$\text{Washington: } R_{876 \text{ nm}} = -8 \pm 3 \times 10^{-8}$$

$$\text{Oxford: } R_{648 \text{ nm}} = +10 \pm 8 \times 10^{-8}$$

where the quoted statistical error represents 2 s.d. There are, however, also systematic effects which we believe do not exceed $\pm 10 \times 10^{-8}$, but which are not yet fully understood. Within this systematic uncertainty the above results are each consistent with $R = 0$.

We conclude from the two experiments that the optical rotation in bismuth, if it exists, is smaller than the values -3×10^{-7} and -4×10^{-7} predicted by the Weinberg-Salam model plus the atomic central field approximation. Indeed Khriplovich⁷ has argued that the approximate theory used does overestimate R by a factor of ~ 1.5 . Lack of experience of this type of calculation means that more theoretical work is required before we can say whether or not the neglected many-body effects in the atomic calculation would make R consistent with the present experimental limits.

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General relativistic incompressibility

SIXTY years ago, Schwarzschild¹ developed what he, and others through the years, regarded as the general relativistic solution for a static, spherically symmetric, incompressible fluid sphere with equation of state $\rho = \rho_0 = \text{constant}$. Others have rejected the incompressibility interpretation of this solution including Eddington² who regarded $\rho - 3p = \text{constant}$ as the incompressible equation of state. We present here a new definition of incompressibility, consistent with basic principles of relativity. We then consider the natural generalisation of the Schwarzschild equation of state to general relativity, namely the constancy of proper density, and find that this approximates a truly incompressible fluid more closely than that presented by both Schwarzschild and Eddington. This could have interesting consequences in astrophysics since the maximum value of gravitational redshift, as well as the upper limit to the mass of a

neutron star, are larger for this model than for the Schwarzschild model, which is traditionally considered.

For the Schwarzschild solution, the upper limit for $2m/r_0$ is 0.889 because at this value the pressure becomes infinite at $r = 0$ (r is the Schwarzschild radial coordinate, m is the total energy of the body, $r = r_0$ at its surface and $G = c = 1$). Thus, for this case, the ultimate limit for static bodies, $2m/r_0 = 1$ cannot be approached. It has also been shown³ that under the seemingly natural restriction for normal bodies, $dp/dr \leq 0$, the maximum value for $2m/r_0$ which can be attained, irrespective of the equation of state, is 0.889, that is, in the Schwarzschild body. Moreover, this value corresponds to a maximum gravitational redshift of $z = 2$, and for some time, when the redshifts of quasi-stellar objects were piling up to $z = 1.95$, the situation was tantalising indeed. Since then, however, the z values of quasi-stellar objects have easily broken the $z = 2$ barrier. Also, ultradense matter raises the controversial question regarding the speed of sound exceeding the speed of light⁴⁻⁶.

In recent years, there has developed a growing realisation that $\rho = \text{constant}$ does not represent a constancy of physical energy density because of the role of gravitational binding energy⁷— ρ represents total energy density excluding gravitational energy.

True incompressibility, an idealisation in the realm of classical physics, is an even more remote concept in general relativity, because its realisation is already precluded in special relativity. Complete rigidity implies the instantaneous velocity of propagation of interactions. Instead, we propose the following definition of incompressibility.

A static, spherically symmetric body is incompressible if its equation of state is such that the pressure becomes infinite at $r = 0$ precisely when the limit $2m/r_0 = 1$ is reached.

Surely this represents the ultimate stiffness of matter which can be envisaged, and it can be envisaged only as a limit; it cannot be attained⁸, which is all to the good.

For physically reasonable equations of state, how close can matter come to being incompressible? We will consider this question in conjunction with our definition: how close can a static body come to having $2m/r_0 = 1$?

We first consider Eddington incompressibility. Letting $\rho - 3p = B = \text{constant}$, we readily solve for one component of the metric

$$ds^2 = e^{\nu} dt^2 - e^{\lambda} dr^2 - r^2 (d\theta^2 + \sin^2\theta d\phi^2)$$

in terms of pressure. From the Einstein field equations

$$p' = -\frac{\nu'}{2}(B + 4p); \quad p = \frac{Ke^{-2\nu} - B}{4}$$

where the prime $\equiv d/dr$ and K is the constant of integration.

Unfortunately, we then learn that the coupling of the equations for density and pressure which is demanded by the Eddington equation of state, leads to far more difficult differential equations for ν and λ themselves. The equation for ν is

$$CKe^{-2\nu} \left(\nu'' - (\nu')^2 - \frac{6\nu'}{r} - \frac{6}{r^2} \right) + CB \left(-\nu'' - (\nu')^2 + \frac{2}{r^2} \right) - \frac{4\nu'^2}{r^2} - \frac{4\nu''}{r^2} - \frac{8\nu'}{r^2} = 0; \quad C = -8\pi G$$

This is to be compared to the Schwarzschild equation of state $p = p_0$ which yields

$$C\rho_0 r^2 = (e^{-\lambda} r)' - 1; \quad e^{-\lambda} = 1 + \frac{C\rho_0 r^2}{3};$$

$$rDe^{-\nu/2} = e^{-\lambda} \nu' - (e^{-\lambda})'$$

With $e^{-\lambda}$ known, this easily leads to the solution for ν .

Nevertheless, we now have computers which were unavailable to Eddington. The numerical solutions have revealed that, at every stage, the Eddington equation of state corresponds to a body which is less stiff than the Schwarzschild body of equal mass and radius. This is consistent with Buchdahl's proof³ because

$$\frac{dp}{dr} = 3 \frac{dp}{dr} < 0$$

We feel that both the Schwarzschild and Eddington equations of state suffer from the deficiency of being too closely wedded to classical concepts. Relativistically, all energy, including gravitational energy, contributes to the total mass of a body, and in the extremely strong gravitational fields here considered, where bodies are close to their gravitational radii, gravitational energy is very significant indeed. In this respect, we now consider the natural general relativistic generalisation of the Schwarzschild equation of states.

The total relativistic mass of a body can be expressed as

$$M = \int_0^{r_0} \rho(r) 4\pi r^2 dr$$

This is found by matching to the exterior Schwarzschild metric. Since the integration is not over proper physical volume, clearly $\rho(r)$ does not represent proper energy density. If, however, we write

$$M = \int_0^{r_0} \frac{\rho(r)}{\sqrt{g_{rr}}} 4\pi r^2 \sqrt{g_{rr}} dr$$

we recognise $\rho_{\text{proper}} = \rho(r)/\sqrt{g_{rr}}$. In fact, in the limit of weak fields, it is readily shown that this modification accounts for gravitational binding energy⁷.

Thus, to generalise the Schwarzschild equation of state

$$\rho_{\text{proper}} = \frac{\rho(r)}{\sqrt{g_{rr}}} = \text{constant}$$

or

$$\rho(r) = A e^{\lambda/2}; A = \text{constant}$$

In conjunction with the Einstein field equations, this yields

$$CAe^{\lambda/2} = e^{-\lambda} \left(\frac{1}{r^2} - \frac{\lambda'}{r} \right) - \frac{1}{r^2}$$

$$Cp = \frac{1}{r^2} - e^{-\lambda} \left(\frac{1}{r^2} - \frac{v'}{r} \right)$$

$$Cp = e^{-\lambda} \left[\frac{v'\lambda'}{4} - \frac{(v')^2}{4} - \left(\frac{v'}{2r} - \frac{\lambda'}{2} \right) - \frac{v''}{2} \right]$$

These equations were solved by numerical integration. The program was verified by solving the Schwarzschild equations, which led to results in agreement with the analytical solution to five significant figures. The results of the computer analysis of the equations are shown below. The equations were integrated outwards with infinite central pressure, and the radius of the body was determined by the condition that the pressure vanishes at the surface.

The graph compares pressure with e^{λ} rather than with radius because

$$(e^{\lambda})_{\text{surface}} = \left(1 - \frac{2m}{r_0} \right)^{-1}$$

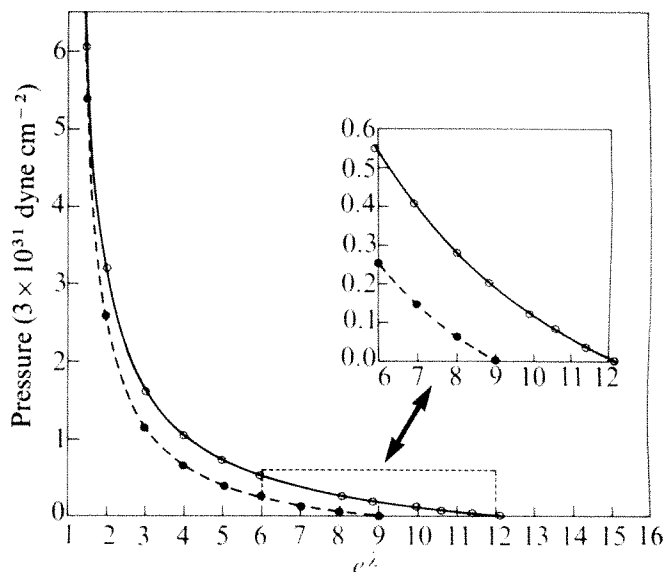


Fig. 1 Comparison of the behaviour of the constant $-\rho_{\text{proper}}$ solution (—) with that of the Schwarzschild solution (---).

yields $2m/r_0$ directly, obviating the necessity of integrating to find the total mass of the system. The Eddington solution has not been analysed for infinite central pressure. From our previous discussion, however, the Eddington equation of state corresponds to matter which is less stiff than that of Schwarzschild. From Fig. 1 $(e^{\lambda})_{\text{surface}} = 12.1$ for ρ_{proper} constant, corresponding to the value $2m/r_0 = 0.917$. Thus, by our criterion, we have material which is stiffer than in Schwarzschild's and Eddington's work. Moreover, it implies a larger maximum gravitational redshift ($z = 2.48$) of spectral lines from the surface of a star. Although it represents an improvement over the Schwarzschild limit, redshifts have been observed to exceed this value⁸ and hence this alone would not suffice to provide an alternative hypothesis to the cosmological interpretation of QSOs.

Eddington's condition is equivalent to the classical concept of incompressibility because classically, for a homogeneous substance, mass and particle density are proportional. The Schwarzschild condition is a generalisation of this concept to special relativity since it takes into account changes in mass from motion, and the $\rho_{\text{proper}} = \text{constant}$ condition, incorporating gravitational energy as well, is a further generalisation of this same concept. It is not surprising, therefore, that among the three, the Schwarzschild fluid should be stiffer than that of Eddington, and the constant $-\rho_{\text{proper}}$ fluid stiffest of all.

As a final note, it is worth mentioning that Bondi⁹ went further still, achieving $2m/r_0 = 0.970$ under the assumptions $\rho > 0, p > 0$. His solution, corresponding to a "thin shell of matter containing a space stuffed full with density-free pressure", is, however, entirely unphysical.

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Is the velocity of P_n an indicator of Q_a ?

IN a recent paper Booth *et al.*¹ showed that short period teleseismic P waves are recorded with relatively small amplitudes at long range seismic measurement (LRSM) stations in the western United States compared with those in the eastern half of the country. This is in agreement with the results of Jordan *et al.*² and several more recent studies. Booth concluded that this might be because the upper mantle anelastic quality factor Q_a is higher in the east, and consequently waves passing through it suffer less absorption.

The hypothesis has important consequences for the measurement of magnitude (m_b) and the proposal made by the United States and the Soviet Union to limit the size of their nuclear weapon tests stimulated further investigations of the idea. There is not an obvious way of doing so because opportunities to make reciprocal station-source experiments (firing at one station and recording at another, then firing at the second and recording at the first) are few. Would other geophysical quantities provide an indicator of Q_a in the upper mantle?

The velocity of the Moho refracted wave, P_n , is an obvious candidate; Herrin and Taggart³ have proposed that the variation in velocity of P_n might be due to fundamental differences between east and west in the upper mantle of North America. Evernden⁴ used P_n velocities to improve the estimates of magnitudes of near and near-regional earthquakes, and Alsop⁵ estimates Q_a in the uppermost mantle of the United States with the help of P_n waves.

The result of plotting Herrin and Taggart's observations³ of P_n velocity against the average short period P-wave amplitude residuals for stations overlying a particular P_n velocity is illustrated in Fig. 1. The two horizontal lines are simple averages. Cleary's⁶ data from the homogeneous (LRSM) network of stations are included with those of Booth *et al.* All five observations at 8.15 km s^{-1} are in Wisconsin and Minnesota where according to Herrin and Taggart, the P_n velocity is not well determined. (Note, however, that Smith *et al.*⁷ give a P_n velocity of 8.07 km s^{-1} for this area and that this eliminates the anomalous value.)

The correlation illustrated in Fig. 1 establishes an empirical relationship between the velocity of P_n and amplitude residuals

in North America. As it seems reasonable to suppose that the amplitude variations are caused by Q_a variation, we conclude that Q_a and the velocity of P_n are correlated. Archambeau *et al.*⁹ have already implied a relation between upper mantle P velocities and Q_a . The work which is developing from these key observations reported in this note indicates that such a relationship does exist, and that useful estimates of effective Q_a of the upper mantle and of amplitude (magnitude) residuals can be made wherever there are reliable measurements of P_n velocity. Since P_n velocities are available from most parts of the world it is possible to improve the accuracy of estimates of seismic yields of underground explosions. The more fundamental investigations into the physical significance of the observations are being pursued.

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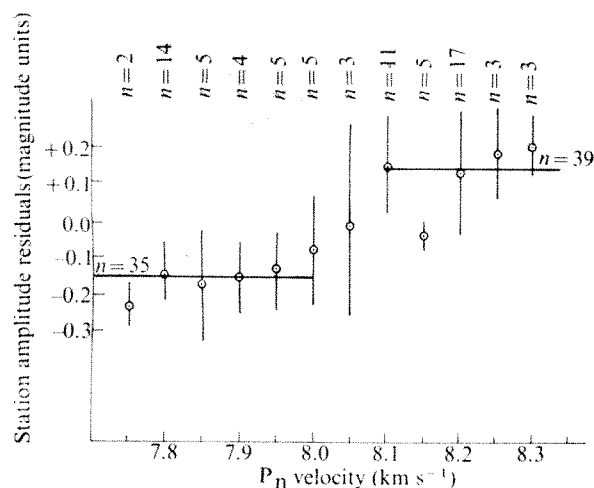
Use of phototransfer for the anomalous fading of thermoluminescence

THE occurrence of anomalous fading of thermoluminescence (TL) in minerals typical of the inclusions found in archaeological pottery has been reported¹. This effect leads to an erroneous evaluation of the archaeological age using conventional TL methods. Consequently it introduces serious problems in the dating of such materials. Here the phototransfer technique (re-excited glow)² was applied. The results indicate that in some materials anomalous fading may be circumvented, or at least substantially reduced. Of the materials of interest in TL dating that display anomalous fading (zircon, fluorapatite and plagioclase feldspars labradorite, andesine and bytownite), fluorapatite and zircon³ were found to be the most promising for the application of the phototransfer technique.

The samples tested (from the same specimen as used by ref. 1) were in the form of loose, crushed, grains and their TL was observed with apparatus that has been described by Aitken and Fleming⁴. Before the experiments these materials were annealed for 15 min at 700°C in an atmosphere of oxygen-free nitrogen. Ultraviolet illumination (usually 1-min exposure) of the samples was made at room temperature (RT) by means of a 300-W xenon lamp, the light from which was passed through a f/4.0 grating monochromator. A $^{90}\text{Sr}/^{90}\text{Y}$ β source was used for the irradiations.

The phototransferred TL (PTTL) was observed using the following procedure: first, irradiation at RT and subsequently erase TL to 500°C ; second, illumination with monochromatic light at RT and third the observation of re-excited TL by means of a glow curve of 500°C . The re-excited glow curves arising from phototransfer, obtained for fluorapatite and zircon are shown in Fig. 1. They are compared with the glow curves obtained after a short β

Fig. 1 Relationship between average station amplitude residuals and P_n velocity. Horizontal bars are obtained by summing all individual station amplitude residuals values $> 8.1 \text{ km s}^{-1}$ and all $< 8.0 \text{ km s}^{-1}$ and dividing by n the total number of observations in each velocity range.



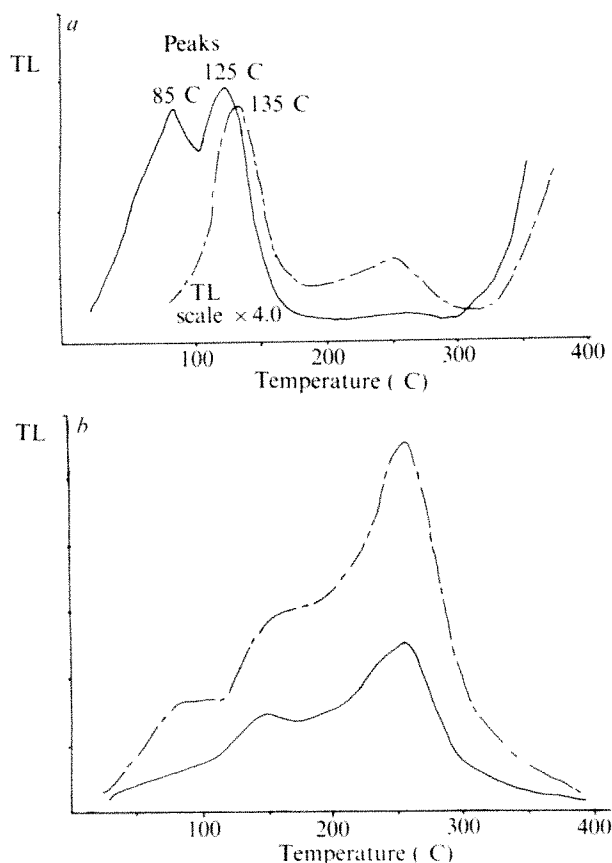


Fig. 1 TL glow curves: (a), zircon and (b) fluorapatite. In both *a* and *b*, — — — represents a typical PTTL glow curve, — — — β -induced TL.

irradiation. In the case of zircon, the re-excited glow curve after ultraviolet illumination is different from that obtained after β irradiation; a peak is observed at 135°C which is close to the dominant peak at 125°C that is induced by β rays.

From the observations of PTTL following drainage to different maximum temperatures, donor levels (populated traps) have associated TL peaks in the region 500–700°C in the case of fluorapatite and in the region 620–700°C for zircon. We tested these donor levels for anomalous fading—samples of zircon and fluorapatite were given a dose of irradiation of ~ 25 krad and stored together with unirradiated samples for periods up to six months. The transferred TL from the irradiated samples was compared with the transferred TL obtained from the previously unirradiated samples that were β irradiated with the same dose just before measurement. Table 1 lists the results of the fading tests for the zircon and fluorapatite samples. In the case of zircon the donor levels associated with the 320-nm illumination were found not to fade and those levels associated with other wavelengths (280 and 240 nm) show some loss. Detailed investigation of the variation of transferred TL with wavelength of illumination³ indicates that there may be two types of centre involved in the transfer, and it appears that one of them does not fade.

Table 1 Long term fading test: % loss of PTTL (experimental error $\pm 3\%$)

Sample Illumination	Test period (months)	% loss transfer 320 nm	% loss transfer 280 nm	% loss transfer 240 nm
Zircon	6	0	15	20
Fluorapatite	3	3	No data	
	5	23		

The results for fluorapatite were less conclusive and the inconsistency (see Table 1) of 3% and 23% fading after 3 and 5 months storage respectively is to be investigated. As a comparison with conventional TL, tests for anomalous fading of the β induced glow curve ($< 500^\circ\text{C}$) were carried out. For storage after irradiation (in the dark, at RT) for periods of 1 h (zircon) and 0.5 h (fluorapatite), the glow curves showed $\sim 15\%$ fading in the high temperature region ($> 150^\circ\text{C}$) that is, where thermal untrapping had not occurred. Similar results were obtained with PTTL. The observations of the fading of the β -induced TL are in agreement with the findings of Wintle⁴, who has found that the anomalous fading of TL is not necessarily short lived; Fig. 2 shows results from her work for fluorapatite and zircon. These graphs indicate that, using conventional TL, extensive loss is to be expected for storage over a few months in the case of fluorapatite though less, but still serious, fading in the case of zircon.

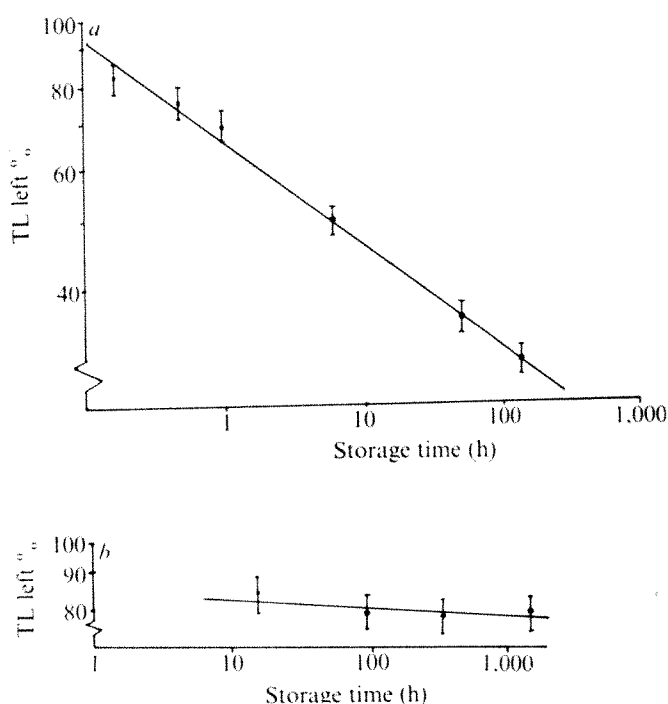


Fig. 2 Anomalous fading of TL. % TL left at 270°C coordinate (fluorapatite (a)) and at 300°C coordinate (zircon (b)) against storage time, at 10°C (log/log plot).

For the purpose of dating, the growth in intensity of transferred TL with dose accrued by the sample (referred to as the 'growth characteristic') has also been investigated. Minerals such as zircon and apatites have high uranium and thorium content; typically 10^2 – 10^3 p.p.m. Consequently the effective internal radiation dose over archaeological time is² in the region of tens of kilorads. For zircon and fluorapatite it has been found that the growth characteristic is linear up to 20 krad of accrued dose (see Fig. 3) for 320-nm illumination. Other wavelengths have also been investigated³. The non-zero intercept on the equivalent dose transferred axis results from a residual ultraviolet response, which in the case of zircon is significant. (The 'residual ultraviolet' excited TL refers to the glow curve obtained after ultraviolet illumination where the sample had not been irradiated since annealing to 700°C.) Investigation of the wavelength dependence of PTTL and the residual ultraviolet excited TL indicates, however, that the situation should be improved for illumination with longer wavelengths. This and further work on the transfer properties of zircon and fluorapatite are to be reported elsewhere.

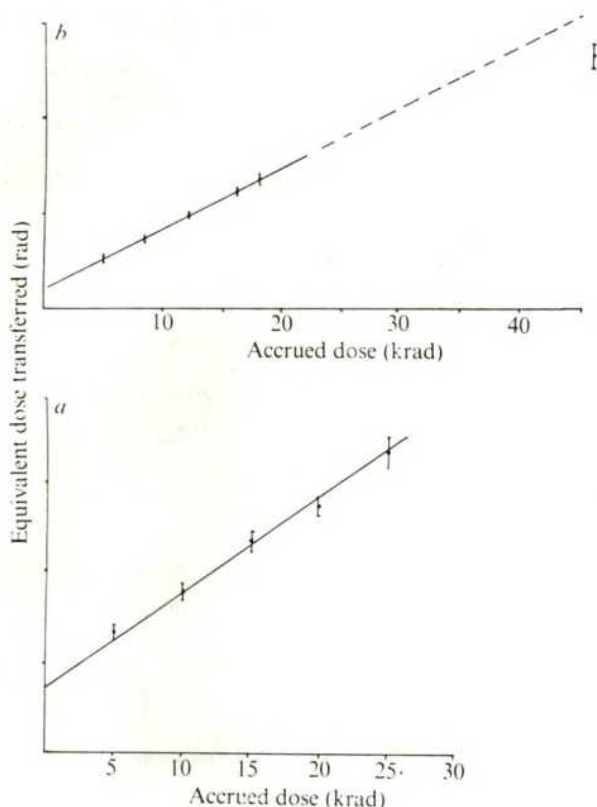


Fig. 3 Growth characteristics for (a) zircon and (b) fluorapatite under 320-nm (5 nm B-P) illumination. The 'equivalent dose transferred' (EDT) refers to the β dose required to be administered to the sample to produce a TL peak height that is equivalent to that of the PTTL peak. In the case of fluorapatite the 250 °C peak was used and for zircon the 125 °C β -induced peak was used to represent an EDT for the 135 °C ultraviolet-excited peak.

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Quantitative approach to molecular resolution electron microscopy

MANY attempts have been made to resolve individual atoms as ions, in molecules, and in crystals using conventional and scanning transmission electron microscopy^{1–5}. The results have been presented in the form of photographs for essentially qualitative assessment. In order to put the technique on a quantitative basis, we have analysed images of isolated molecules on thin supporting films using a digitising microdensitometer. We report here the results for a particular test molecule with a readily identified structure containing one heavy atom ($Z = 80$) for reference and two medium ones ($Z = 35$) for

determining the reliability threshold. As yet, images of lighter atoms ($Z = 16$) have been obtained only after extensive processing¹, which impedes quantitative assessment.

We used a JEM 100 C microscope (JEOL Ltd, Japan) with voltage 120 kV, tilted beam dark field operation, objective aperture for spatial periods down to 0.24 nm, negative defocusing close to the Scherzer condition, and calibrated magnification 830 k. We chose Kodirex film (Kodak-Pathé, France) for the recording, as it has appropriate sensitivity, modulation transfer function (MTF), and detective quantum efficiency (DQE) characteristics. The specimen consisted of Merbromin ($C_{20}H_{10}Br_2HgO_6$) (K & K: ICN Life Science Group, USA) molecules deposited from solution in water on the support film. Figure 1 shows the probable structure determined by modelling: the separations of the mercury and bromine atoms are about 1.1, 1.0 and 0.6 nm. The support was a 400-mesh copper grid coated with Formvar, a holey carbon film, and an ultrathin (≤ 1.5 nm) amorphous boron layer. We found that the boron layer was stronger and more uniform over the holes than carbon ones, and gave less background noise. Bromine atoms are usually sufficiently well resolved against uniform regions of the support film to present few problems. We deliberately chose a molecule close to a non-uniform region in order to demonstrate the danger of interpreting from 'improved' photographs, and to demonstrate that the quantitative analysis of the original recording yields a measure of its reliability. Figure 2 shows two enlargements made from the same part of the original. Fig. 2a was printed conventionally. (The process could not resolve the low contrast details visible in the negative.) Fig. 2b was obtained by copying with four times magnification on high gamma duplicating film and then printing on hard paper. It reveals the bromine atoms;

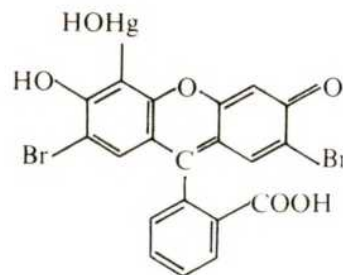
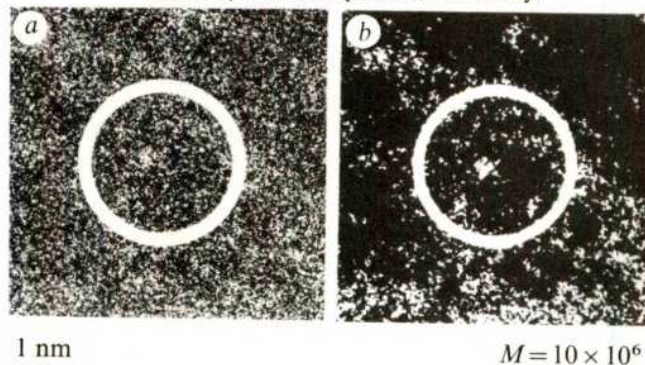


Fig. 1

but the granular structure and the lack of intermediate tones makes it difficult to distinguish the molecule from the noise. There are other processing methods, which yield cleaner pictures¹; but the probability of misinformation is greater.

In our quantitative approach, we first examined the original recording using a low magnification optical microscope with numerical aperture sufficiently large for the range of spatial periods. We then evaluated likely regions using a microdensitometer. This was a flat-bed model which

Fig. 2 Triad of mercury and bromine atoms in merbromin molecule; b has been processed for clarity.



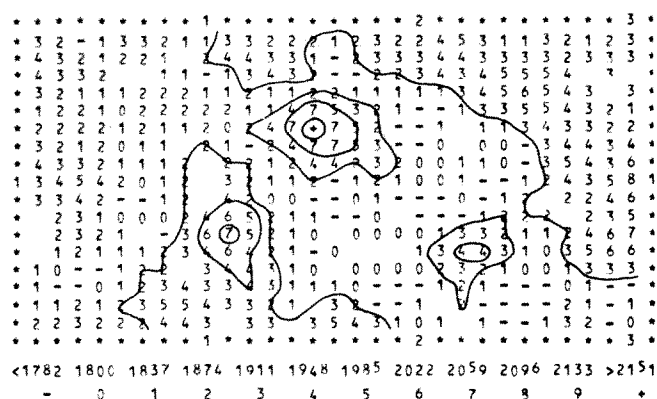


Fig. 3

had been modified in the laboratory for scanning and digitisation. A $(25\text{-}\mu\text{m})^2$ sampling aperture yielded reasonably constant MTF for spatial periods down to $\sim 0.15\text{ mm}$, and that we found adequate for Kodirex. We chose the sampling step somewhat smaller than half the minimum spatial period for the microscope, $80\text{ }\mu\text{m}$ corresponding to 0.096 nm . The data were tabulated by computer. A crude contour map is shown in Fig. 3: each number indicates the highest of ten contour levels which passes within half a spacing of a grid point. (We found it necessary to use a second evaluation for the map with a larger sampling aperture so that the background fluctuations were reduced by MTF roll-off.) The data from single line scans through the atoms are shown in Fig. 4. We considered it inappropriate to fit curves through the points, as that was not particularly informative in view of the sampling process. We also found that the measured optical densities, D , were not very helpful, and we decided to refer the values to the mean \bar{D} (horizontal line) and the root mean square fluctuation \bar{D} for background scans on either side of the molecule. The resulting local signal-to-noise ratios $(D-\bar{D})/\bar{D}$ were 3.8 and 2.0, 1.8 for the mercury and bromine atoms. The mean Hg/Br signal ratio for several evaluations with different specimens was 1.9, suggesting reasonable prospects for heavy/light atom discrimination. The variance within the (admittedly) small sample was disconcerting, implying poorer reliability than we had supposed from the visual assessment.

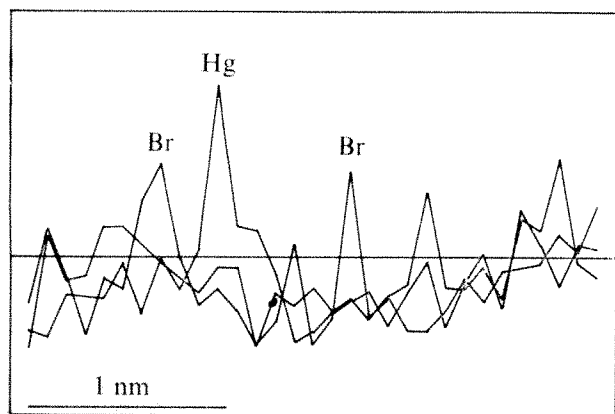


Fig. 4

The scan profiles can be misleading: for example, the peaks to the right of the molecule may be interpreted as other atoms. Careful analysis of the patterns in two directions normally separates the useful information from the "noise"; but that clearly relies on previous knowledge of the structures. The widths of the profiles are no guide for the resolution attainable. We believe that the minimum workable separation is significantly smaller than twice the limiting spatial period; but, unfortunately, it is certainly larger than the atomic bond distances. (We are now trying molecules with smaller spacings.)

We are sufficiently encouraged by the results for uranium, mercury, barium, iodine, strontium⁵ and bromine atoms to continue the search downwards. In view of the background fluctuations, we are doubtful of the prospects for light atoms, but we remain hopeful for chlorine ($Z = 17$). In another direction, we are collaborating in an application to tag subunits of large biomolecules. We are also developing more practical techniques of image analysis using a minicomputer with a graphics display terminal.

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Low temperature specific heat of glasses and amorphous solids

IN a previous paper¹ we attributed the anomalous low temperature thermal properties of amorphous materials to the inherent nature of the amorphous state, conceived of as the crystalline state saturated with the most favoured dislocations². Using Granato's vibrating string model³ of a dislocation, an estimate of the excess specific heat of $\text{Pd}_{0.775}\text{Cu}_{0.06}\text{Si}_{0.165}$ was provided. The purpose of the present note is to clarify several points in our earlier paper.

The experimental value^{4,5} of the specific heat of glassy $\text{Pd}_{0.776}\text{Cu}_{0.06}\text{Si}_{0.165}$ previously quoted is actually the electronic specific heat of the crystalline solid and not the linear contribution associated with the amorphous state. For the anomalous linear region of amorphous $\text{Pd}_{0.775}\text{Cu}_{0.06}\text{Si}_{0.165}$ the data of Golding, Bagley and Hsu¹ indicate an upper limit of $C_v/T \approx 1.1 \times 10^4 \text{ erg mol}^{-1} \text{ K}^{-2}$.

As the previous calculation for the linear specific heat of amorphous $\text{Pd}_{0.775}\text{Cu}_{0.06}\text{Si}_{0.165}$ used weighted averages for the parameters involved, it is felt that a revised calculation using experimentally determined values is in order. The pertinent values determined by Golding, Bagley and Hsu¹ are $\rho = 10.52 \text{ g cm}^{-3}$, $G = 3.48 \times 10^{11} \text{ dyne cm}^{-2}$, and $\theta = 252 \text{ K}$. Noting that the saturation condition corresponds to a dislocation density $\Lambda = (2r_c^2/3)^{-1}$ where r_c is the core radius, then saturation occurs for $\Lambda = 7.9 \times 10^{13} \text{ cm}^{-2}$ (a weighted average of $4.1 \times 10^{-8} \text{ cm}$ has been assumed for the lattice parameter). Substituting the above values into the expression for the dislocation contribution to the specific heat.

$$C_v = \frac{\rho \pi^2 \Lambda a^2 N k T}{3 z \theta} \quad (1)$$

now yields $C_v/T = 8.6 \times 10^4 \text{ erg mol}^{-1} \text{ K}^{-2}$, as compared with the experimental value of $1.1 \times 10^4 \text{ erg mol}^{-1} \text{ K}^{-2}$. It must be noted that this is an absolute upper limit on the calculated dislocation specific heat contribution.

Lu and Nelkin⁶ have also examined the low temperature thermal properties of glasses in terms of dislocations, indicating that a dislocation density of 10^{10} – 10^{11} cm^{-2} is required to

explain the linear specific heat (this calculation employed the Lifshitz-Kosevich⁷ model of an edge dislocation) and further speculating that the tunnelling of dislocation cores between equilibrium configurations may be responsible for the phonon scattering (a similar idea has been advanced by Anderson⁸ for a sessile dislocation in a complex lattice). Tunnelling of the cores would be consistent with the two-level systems postulated in the tunnelling states model^{9,10}.

There are difficulties associated with the concept of dislocations in glasses, the validity of the string model at these high densities, and the behaviour of the strain fields at this density. These are all questions which need to be addressed. But Ashby and Logan¹¹ have pointed out that within the framework of the random-network model "dislocation-like" features are possible in glasses and that they may have a very high density. In earlier work Scott and Giles¹² have succeeded in explaining the thermal conductivity of Teflon from 0.17 to 4.0 K on the basis of dislocation scattering of phonons. Further, small-angle X-ray scattering experiments are not inconsistent with the presence of dislocation strain fields in glasses⁶. Caution should also be exercised in strict application of the string model to explain both the specific heat and thermal conductivity of glasses. But in view of the rather interesting, although speculative, implications of these ideas, we feel that the concept of dislocations in amorphous solids is worthy of further examination, especially its compatibility with the tunnelling states model.

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Fig. 1 View of venting St Augustine volcano as the aircraft lines up for a plume penetration.

1976), taken as the National Center for Atmospheric Research (NCAR) Electra aircraft (instrument boom visible to left) prepared to enter the plume on a sampling run, is shown in Fig. 1. During this and other sampling runs ice nuclei in the air were captured on Millipore membrane filters using the NCAR SAMOVAR Air Filtration System⁶.

To reduce the analytical uncertainty that seems to be associated with all methods of membrane ice nucleus analyses, rather than filtering aerosol through single 3.7- or 4.7-cm diameter filters as is commonly done, an array of filters was produced by first exposing a single 28.7-cm square, 0.45 μm pore diameter, Millipore filter supported by a fibre glass backing. This large filter was then sectioned with a circular stainless steel die to produce 25 separate 4.7-cm diameter filter disks all exposed in the same volume of air. Each of the segments is considered to be equivalent to each of the others. Between 0.5 and 5.0 m^3 of air flowed through each filter disk at a rate of 0.15 $\text{m}^3 \text{min}^{-1}$.

One part of the experiment consisted of exposing a large Millipore filter 800 km upwind of the volcano over the Gulf of Alaska in marine air far removed from volcanic and human influence. This air was considered to contain a representative background ice nucleus population for the air subsequently moving on to the coast of Alaska. The first exposure was followed by exposure of a new filter totally within the plume of the volcano on the following day. Other measurements included exposure of a filter in background air and re-exposure of the same filter within the plume. This sequence was followed to observe whether plume effluents deactivated natural ice nuclei. Yet another filter was exposed solely in plume air 16 d after the second major eruption.

The Millipore filter sections were processed in a thermal diffusion chamber identical to the one described by Langer and Rodgers⁷, except for the water vapour source for these studies which consisted of three thermally controlled ice-covered plates instead of ice cubes. From each large filter array, five filter disks were processed at -10 , -15 , and -20 $^{\circ}\text{C}$. To calibrate the system to operate at water saturation, or possibly slight supersaturation, the filter substrate and ice temperatures were adjusted so that a fog, and eventually droplets, appeared on aluminium foil placed in the processing chamber. Further evidence of proper vapour control was seen when water-depleted 'halos' formed around growing ice crystals on the filters being processed; thus it may be suggested that condensation-followed-by-freezing may have been occurring in addition to nucleation by deposition. Typical ice crystal counts per filter were 1–20 at -10 $^{\circ}\text{C}$, 9–90 at -15 $^{\circ}\text{C}$, and 200–800 at -20 $^{\circ}\text{C}$ before

Airborne ice nuclei near an active volcano

ACTIVE volcanoes have been suggested as being sources of atmospheric ice nuclei^{1,2}, the rare yet crucial particles that initiate much of the Earth's precipitation. Other studies indicate that some volcanoes are not adding ice nuclei to the atmosphere or may be deactivating natural ice nuclei^{3–5}. We report here measurements of atmospheric ice nucleus concentrations upwind and within the effluent plume of St Augustine, an actively venting island volcano situated at the base of the Aleutians in the mouth of Cook Inlet, Alaska ($59^{\circ}20' \text{N}$, $153^{\circ}30' \text{W}$). St Augustine erupted violently on January 23, 1976 and then again on February 4, 1976. We find no evidence for ice nuclei production by this volcano. Between and after the major eruptions the volcano actively vented giving off gaseous and particulate matter. Sulphur dioxide concentrations in the plume were $>5 \text{ p.p.m.}$ (J. Moyers, unpublished) and the total particulate loading was $\sim 100 \mu\text{g m}^{-3}$ (W. Zoller, unpublished) during the venting stages. A photograph of the venting volcano (February 1,

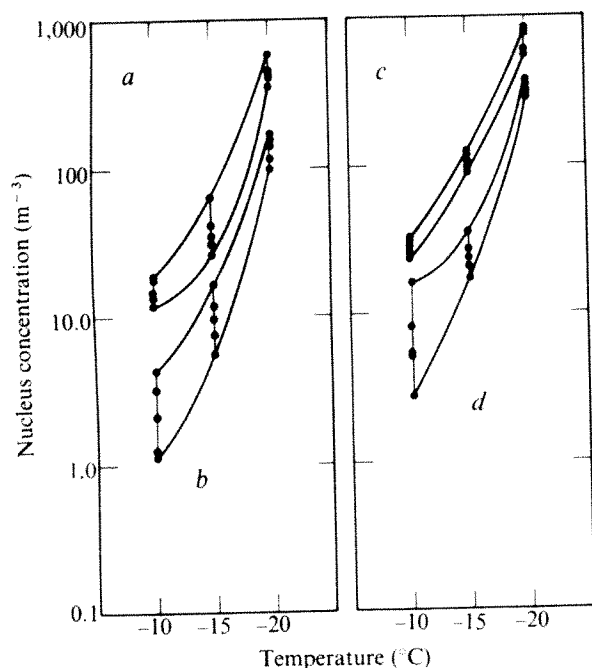


Fig. 2 Atmospheric ice-nucleus concentrations measured upwind and within the plume of erupting St Augustine volcano, Alaska. The solid dots represent the ice nucleus concentrations observed; the trend lines serve merely to bracket the separate ice nucleus concentration sets. *a*, Gulf of Alaska, January 31, 1976; *b*, plume, February 1, 1976; *c*, background and plume, February 1, 1976; *d*, plume, February 20, 1976.

subtraction of background counts (observed on unexposed filters) of 9, 6, and 45 crystals, respectively. The resultant crystal counts were corrected for volume effect⁸ and then normalised to a unit volume of air.

Results from analyses of four filter arrays are shown in Fig. 2 where it can be seen that the air over the Gulf of Alaska sampled on January 31, 1976 (*a*) contained more ice nuclei than did the volcanic plume sampled on the following day, February 1 (*b*). Comparisons between the ice nuclei in the plume air on February 1 (*b*) and the ice nuclei observed on filters exposed to background air followed by re-exposure to plume air (*c*) indicate that slightly higher ice nucleus concentrations were observed in the latter samples. The plume air of February 20 (*d*) contained ice nuclei in concentrations slightly in excess of the background air measured over the Gulf of Alaska (*a*).

All of the ice nucleus measurements shown in Fig. 2 fall within the range of 'typical' ice nucleus spectra observed in 'background' air at other locations around the globe⁹. The concentrations of ice nuclei active at -20°C in the plume on February 20 (*d*) (highest concentrations observed) fall between 560 and 920 nuclei m^{-3} . These values bracket the measurement of 0.7 nuclei l^{-1} active at -20°C measured in the plume of venting Mt Baker (Washington) during March and June 1975 as reported by Radke *et al.*¹⁰.

We suggest, on the basis of our measurements, that the effluent plume of St Augustine was not contributing significantly to background atmospheric ice nucleus concentrations on the days studied. There is some suggestion that the effluent gases may be deactivating natural ice nuclei in some cases as evidenced by relatively low ice nucleus concentrations measured on February 7. If this is so, this deactivation is relatively small compared with the inherent variability of natural atmospheric ice nucleus concentrations generally observed in the atmosphere¹¹. This variability could also explain these lower measurements. Since the particulate output of all the Earth's volcanoes (actively erupting and venting) is estimated to be ~ 0.005 of the total Earth-derived aerosol load¹¹, it is further suggested that

volcanoes venting similarly to St Augustine are probably exerting little influence on local atmospheric ice nucleus concentrations and negligible effects on global atmospheric ice nucleus concentrations. We anticipate measuring ice nucleus concentrations within the plume of an erupting volcano in the coming years.

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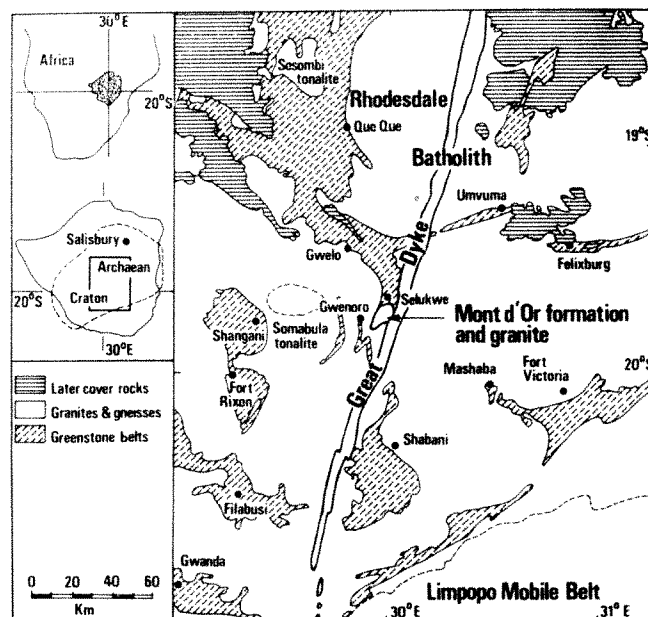
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Early Archaean age for the Sebakwian group at Selukwe, Rhodesia

WE present here the results of a Rb-Sr age and isotope study of granite (about 70% SiO_2) from the north-eastern sector of the Mont d'Or formation in the central part of the Rhodesian Archaean craton (Fig. 1). A nine-point Rb-Sr whole rock isochron (Fig. 2, Table 1) yields an age of $3,420 \pm 60$ Myr, with an initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.711 ± 0.001 (both errors at 1σ). We regard this as the age of intrusion.

The current nomenclature for the subdivisions of Rhodesian greenstone belts invokes three informal lithostratigraphic 'groups', the Sebakwian, Bulawayan and Shamvaian.

Fig. 1 Geological sketch map of part of the Rhodesian Archaean craton, showing locality of Mont d'Or granite.



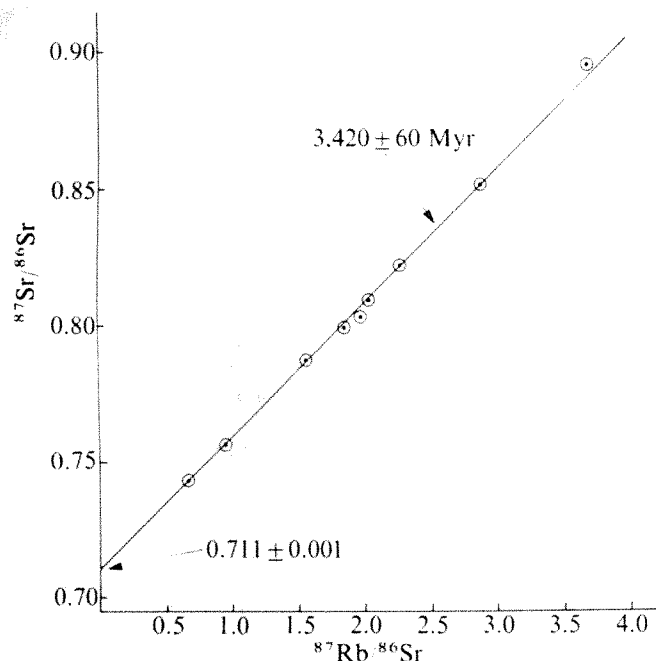


Fig. 2 Rb-Sr whole rock isochron plot for Mont d'Or granite samples. Errors are quoted at the 1 σ level. Error factor required to achieve perfect least squares fit is 2.5. Decay constant for ^{87}Rb is $1.39 \times 10^{-11} \text{ yr}^{-1}$.

The Sebakwian is the oldest, with the younger Bulawayan and Shamvaian groups together constituting the main greenstone belts¹. Age measurements on volcanic rocks from the Bulawayan group indicate an age of eruption of about 2,700 Myr (ref. 2). The Selukwe greenstone belt is assigned to the Sebakwian group on the latest (1971) edition of the geological map of Rhodesia issued by the Rhodesia Geological Survey. The Selukwe area is unique in that the succession is inverted and forms part of the lower limb of a large recumbent fold³⁻⁷. This structure is called the Selukwe nappe.

Table 1 Rb-Sr analytical data for Mont d'Or granite

Sample no.*	$^{87}\text{Rb}/^{86}\text{Sr}^\dagger$	$^{87}\text{Sr}/^{86}\text{Sr}$	Rb(p.p.m.) ‡	Sr(p.p.m.) ‡
1,167	2.04	0.8083 \pm 1	71	101
1,204	1.97	0.8010 \pm 2	66	97
1,223	1.55	0.7873 \pm 1	51	95
1,249	2.88	0.8514 \pm 1	100	102
1,263	1.85	0.7982 \pm 1	96	151
2,123	2.27	0.8218 \pm 1	110	142
2,974	3.68	0.8949 \pm 1	65	52
3,692	0.659	0.7431 \pm 1	42	185
3,724	0.951	0.7561 \pm 1	53	162

*From borehole S636, depths in feet.

† Determined by X-ray fluorescence¹⁰; average precision \pm 1%.

‡ Mass absorption coefficients estimated from background; concentration data \pm 5%.

The Mont d'Or formation consists largely of quartzofeldspathic rocks containing chlorite and sericite. The sericitised nature of many of the rocks has led to dispute concerning the original nature of the formation. Stowe³⁻⁵ favours a largely sedimentary origin and regards the Mont d'Or formation as the lowest (oldest) stratigraphic unit in the nappe structure, but describes the presence of later, small, irregular intrusive bodies of granite. Cotterill⁷, on the other hand, considers that the greater part of the formation consists of a granite which is intrusive into the nappe structure. He recognises meta-sedimentary enclaves within the granite, but regards them as lateral equivalents of the Wanderer formation, the major sedimentary unit within the nappe succession (Table 2).

The difference in interpretation is, in effect, largely one of amount of later granite present. Our samples come from a deep borehole (S636) from the northern part of the area underlain by Mont d'Or formation where both authors agree to the presence of later granite. Thin sections of our samples show quartz, potash feldspar, sodic plagioclase, a small amount of chloritised biotite, and variable sericitisation of the feldspars.

Our results demonstrate a pre-3,400 Myr age not only for the Selukwe greenstone belt (Sebakwian group), but also for the nappe structure itself.

Table 2 Main stratigraphic units of the Selukwe greenstone belt, youngest formation at the top

After Stowe ⁵	After Cotterill ⁷
Small bodies of late granite	Mont d'Or formation (dominantly granite) with intrusive contact
Nappe tectonics	Nappe tectonics
Upper Greenstone formation	Tibilikwe formation (metabasalt with minor banded iron formation)
Wanderer formation	Wanderer formation (conglomerate and grit, overlain by banded iron formation)
Unconformity	Unconformity
Lower Greenstone formation (with serpentinites)	Selukwe Greenstone formation intruded by Selukwe ultramafic formation
Mont d'Or formation (dominantly meta-arenites)	

The eastern side of the Selukwe greenstone belt is cut by the Great Dyke (\sim 2,500 Myr). East of the Great Dyke, the metasupracrustals and chromite-bearing ultramafic rocks of the extension of the Selukwe greenstone belt occur tightly infolded with northerly trending banded gneisses and migmatites. Very similar rocks, again assigned to the Sebakwian group, and also tightly infolded with gneisses on a distinct northerly trend, occur farther east in the Mashaba area^{1,8}. Gneisses from the Tokwe and Shashe rivers near Mashaba have yielded a rather poorly defined age of $3,580 \pm 200$ Myr (ref. 2). East of Mashaba, the Mushandike granite, which intrudes similar gneisses, gave an age of $3,520 \pm 130$ Myr (ref. 9).

Thus our results from the Mont d'Or formation granite are in keeping with the regional picture of a \sim 3,500–3,600 Myr granite-gneiss terrain between Selukwe and Mashaba. In addition, they provide confirmation of the inference by Hawkesworth *et al.*² that the greenstone belt remnants within this terrain are also \sim 3,500–3,600 Myr old. The Selukwe greenstone belt is thus the largest preserved belt of this age known in Rhodesia. The possibility also arises that the Selukwe nappe structure is related to the tectonism which produced the prominent northerly trends in the gneisses between the Great Dyke and Mashaba.

The initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.711 ± 0.001 of the Mont d'Or granite is, as far as we know, the highest value yet recorded for such an ancient granite. The initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios for the \sim 3,500–3,600 Myr gneisses mentioned above are within the range 0.701–0.702. We provisionally interpret the high initial ratio of the Mont d'Or granite as indicating partial melting of earlier sialic crust, which need not necessarily be very much older. Thus, 3,600-Myr-old gneisses from the Shabani area, some 70 km S of Selukwe, have sufficiently high Rb/Sr ratios for their average $^{87}\text{Sr}/^{86}\text{Sr}$ ratio to increase by about 0.005 to 0.010 in as little as 200–300 Myr (S.M. and J.F.W., unpublished). It is possible that the Selukwe area is also underlain by ancient, high Rb/Sr crust of this type.

There is clear evidence for earlier granitic rocks in the pre-Mont d'Or Wanderer formation. Stowe³⁻⁵ and Cotterill^{6,7} record boulders and pebbles of various types of granite and gneiss in the conglomerate of this formation.

These could have been derived by erosion of 3,500–3,600-Myr-old basement similar to that exposed further to the south and east.

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Monitoring the marine environment for mutagens

VARIOUS chemicals found in the environment are mutagenic when tested in an appropriate screening system¹, and some of them are deposited as waste in the seas and oceans, where they may or may not be deactivated. A means of monitoring the marine environment for the presence or absence of mutagens would give some indication of the efficiency of disposal of industrial waste products. In an attempt to minimise the technical problems, we have developed a biological assay for the detection of such pollutants.

Our assay detects mutagens in the tissue of the mussel *Mytilus edulis*, which is widely distributed in the tidal zone

and shallow coastal waters of the northern and southern hemisphere, and has been used in several studies of water pollution and interspecific relationships^{2–5}. Because of its mode of filter feeding and intracellular (phagocytic) digestion, this organism may concentrate marine pollutants within its body tissues and these provide a source of testable material.

Test samples were screened using auxotrophic yeast cultures to detect the induction of prototrophic colonies produced by mitotic gene conversion, a process of genetic change that responds to treatment by mutagens and carcinogens in a nonspecific manner⁶. Extracts were also exposed to cultures of both *Salmonella typhimurium* and *Escherichia coli* carrying genetic markers to detect forward and reverse mutation produced by both base-substitution and frameshift DNA changes.

Yeast cells were incorporated with mussel extracts and minimal selective medium in the presence of sufficient growth supplement to enable the cells to undergo three rounds of cell division. *S. typhimurium* was used in a plate incorporation system, as described by Ames *et al.*⁷. *E. coli* was used in both plate incorporation tests and modified bacterial fluctuation tests, as described by Green *et al.*⁸.

Alcoholic extracts were prepared from mussel samples collected from Plymouth (heavy visual pollution), Mumbles, Caswell Bay and Anglesey (clean water area), all in the United Kingdom. The results of exposure of yeast cultures auxotrophic for histidine and tryptophan to mussel extracts at concentrations up to 4% are shown in Table 1. Significant increases in the production of both histidine and tryptophan prototrophs were produced by mussel extracts from Plymouth, Mumbles and Caswell Bay, whereas no such increases were detectable in the presence of mussel extracts derived from the clean waters of Anglesey. Table 1 also contains the positive results of treatment in identical conditions with ethyl methane sulphonate, and the negative result obtained when alcohol was used as a control. Table 1 also shows the results of mussel extracts (Mumbles) derived from either liver tissue alone or non-liver tissue. When prepared in this manner, most of the genetic activity was found to be due to the non-liver extracts.

Table 1 Induction of mitotic gene conversion by mussel extracts

Treatment	Mean no. of <i>his</i> ⁺ prototrophs per plate	<i>his</i> ⁺ prototrophs per 10 ⁶ survivors ± s.e.	Mean no. of <i>trp</i> ⁺ prototrophs per plate	<i>trp</i> ⁺ prototrophs per 10 ⁵ survivors ± s.e.	% Viability
Control 1	8.6	26.8 ± 4.6	7.4	23.1 ± 4.3	91.4
Control 2	7.8	32.5 ± 5.8	5.2	21.7 ± 4.8	68.6
4% Alcohol control 1	6.5	25.6 ± 5.0	9.4	37.0 ± 6.0	73.1
4% Alcohol control 2	4.2	11.8 ± 3.0	7.6	21.4 ± 3.9	101.4
Plymouth extract 4%	202.6	493.6 ± 21.3	154.8	463.5 ± 18.6	95.4
Mumbles extract 4%	71.2	323.6 ± 19.2	85.3	387.7 ± 20.6	62.9
Caswell Bay 4% extract	69.0	276.0 ± 16.6	69.2	276.8 ± 16.6	71.4
Anglesey 4% extract	4.7	18.3 ± 4.2	7.6	29.6 ± 5.4	73.4
Mumbles liver 4% extract	10.8	40.0 ± 6.1	11.4	42.2 ± 6.3	77.1
Mumbles non-liver 4% extract	66.0	307.0 ± 18.9	123.2	573.0 ± 25.8	61.4
Ethyl methane sulphonate (1 µg ml ⁻¹)	284.7	900.9 ± 26.7	346.2	1,095 ± 29.4.7	90.3

Mussel samples were prepared by the extraction of 50 shelled mussels in 100 ml of 95% ethanol in a Waring blender. After centrifugation at 5,000g, extracts were filter sterilised and stored at 4 °C. Stationary phase cultures of the yeast strain *JDI*⁹, auxotrophic for histidine and tryptophan were suspended in saline at a concentration of 10⁷ cells per ml. Samples of mussel extract (up to 4%) were added to yeast minimal medium at 45 °C together with yeast cells at a concentration of 35 × 10³ cells per ml for detection of histidine independent prototrophs and 35 × 10³ cells per ml for detection of tryptophan-independent prototrophs and poured into 9-cm plastic Petri dishes. The culture medium was supplemented with 20 µg ml⁻¹ tryptophan and 0.1 µg of histidine for detection of histidine-independent prototrophs and with histidine (20 µg ml⁻¹) and 0.1 µg of tryptophan for detection of tryptophan independent prototrophs. These supplements enabled auxotrophic cells to undergo three cell divisions in the presence or absence of the mussel extract. All plates were grown in the dark at 28 °C and scored after 9 d of incubation. The possible nutrient effects of the Plymouth mussel extracts on auxotrophic yeast cultures were determined in two ways. (1) After incubation of plates as described above, disks of agar 9 mm in diameter were removed from areas of the selective plates showing background growth, but no large prototrophic colonies. Agar disks sampled from 10 plates per treatment were added to 20 ml of sterile saline and the yeast cells contained in the disks were suspended by sonication. After appropriate dilutions, the saline suspensions were plated on nutrient agar and viable cells were counted after 5 d of incubation at 28 °C. Comparisons of the viable cells contained in the background agar of both treated and untreated cultures indicated that there was no evidence of further growth of auxotrophic yeast cells in solid medium in the presence of mussel extract. (2) Samples of mussel extract were added to liquid minimal medium containing 10⁵ yeast cells per ml and the cultures were aerated at 28 °C for 24 h. Cell growth was determined by counting with a haemocytometer. In no case could we detect increased growth of cells in liquid medium in those cultures containing mussel extracts.

Table 2 Mutation induced in *E. coli* tester strains by MMS and mussel extracts as shown by the fluctuation test

<i>E. coli</i> strain	Locus	Treatment	No. of tubes sampled	Control tubes	Positive test	Significance (probability)
343/113	<i>arg₅₆</i>	MMS	50	21	39	$P < 0.001$
		Mumbles (L)	50	24	34	$P < 0.05$
		Mumbles (NL)	50	24	41	$P < 0.001$
343/113(R46)	<i>arg₅₆</i>	MMS	50	28	48	$P < 0.001$
		Mumbles (L)	50	35	41	NS
		Mumbles (NL)	50	35	45	$P < 0.01$
343/113(R46)	<i>galR₁₈^s</i>	MMS	100	60	99	$P < 0.001$
		Mumbles (L)	100	60	80	$P < 0.001$
		Mumbles (NL)	100	60	95	$P < 0.001$
343/113(R46)	<i>arg₅₆</i>	Burry Estuary (L)	50	13	20	NS
		Burry Estuary (NL)	50	13	31	$P < 0.001$
		MMS	50	22	32	$P < 0.05$
343/113	<i>lys₆₀</i>	Mumbles (L)	50	27	33	NS
		Mumbles (NL)	50	27	29	NS
		MMS	100	48	75	$P < 0.001$
343/113(R46)	<i>lys₆₀</i>	Mumbles (L)	100	65	67	NS
		Mumbles (NL)	100	65	68	NS
		Burry Estuary (L)	50	25	31	NS
343/113(R46)	<i>lys₆₀</i>	Burry Estuary (NL)	50	25	28	NS
		MMS	50	20	33	$P < 0.01$
		Mumbles (L)	50	13	18	NS
WP2 <i>uvrA</i>	<i>trp</i>	Mumbles (NL)	50	13	18	NS
		MMS	100	54	87	$P < 0.001$
		Mumbles (L)	100	48	59	NS
WP2 <i>uvrA</i> (R46)	<i>trp</i>	Mumbles (NL)	100	48	70	$P < 0.005$
		Burry Estuary (L)	100	54	56	NS
		Burry Estuary (NL)	100	54	63	NS

Fluctuation tests were carried out, with minor modifications, as described by Green *et al.*⁸. Overnight cultures of the *E. coli* tester strains were prepared in supplemented Davis-Mingoli minimal medium. After washing twice in saline, the cells were resuspended in saline at a concentration of 5.0×10^7 cells per ml. To 100 ml of Davis-Mingoli basal salts was added 10 ml of 40% glucose (for MMS), 0.1 ml of tryptophan (for liver tissue extracts) solution containing 200 µg per ml (for fluctuation tests involving tryptophan auxotrophs only) or 0.1 ml of lysine (for liver extracts) solution containing 200 µg per ml (for lysine auxotrophs only), 0.1 ml of washed cells, and in the case of the test treatments, either MMS to a final concentration of 1 µg per ml or 0.1 ml of alcohol mussel extract (liver or non-liver). Control experiments were conducted as for the test treatments but omitting MMS or mussel extracts. Controls for experiments involving mussel extracts also contained 0.1 ml of 96% ethanol. Each treatment was dispensed in 2.0-ml samples into 50 small test tubes and incubated at 37 °C. Once the auxotrophic bacteria had exhausted the small amount of supplement present, only prototrophic revertants continued to grow. From 2 d onwards, tubes in which mutation to prototrophy had occurred became turbid, while the other tubes remained relatively clear. The number of turbid tubes was determined routinely after 3 d. *E. coli* strains 343/113 and 343/113 *lys₆₀* were gifts from Dr G. Mohn and *E. coli* WP2 *uvrA* was a gift from Dr M. H. L. Green. To investigate the possible presence of free amino acids in mussel extracts that would increase spontaneous mutation rates by enhancing cell division, viable counts were made from individual cultures of *E. coli uvrA trp* (R46) obtained from fluctuation experiments with and without Mumbles mussel extract. It was found that the median number of bacteria per clear tube, that is tubes that did not contain *trp*⁺ revertants, was unaffected by the presence of mussel extract, and therefore Mumbles mussel extract did not enhance the growth of *trp*⁻ cells when the tryptophan supply has become exhausted. For tests involving the *galR₁₈^s* system, galactose replaced glucose in the fluctuation medium, also as this marker gives a high spontaneous number of turbid tubes each treatment was dispensed in 1.0-ml samples into 100 test tubes. The *arg₅₆* mutation is leaky and as a result some residual growth was observed even in the absence of arginine and therefore trace amounts of arginine were not added when it was used. L, liver, NL, non-liver, NS, not significant.

Table 2 shows the effects of both methyl methane sulphonate (MMS) and mussel liver and non-liver extracts on various strains of *E. coli* as measured by fluctuation tests. *E. coli* 343/113 *arg₅₆*, *nad₁₃₁*, *thi*, *lys₆₀* is a K12 strain developed by Mohn *et al.*¹⁰ for the reliable detection of different types of mutagen, utilising markers in the same genetic background. The *arg₅₆* mutation can be reversed, resulting in arginine prototrophy, by both base-change and frameshift mutagens; *gal R₁₈^s* is a forward mutation and the ability to use galactose as a carbon source can also be restored by both types of mutagen. *lys₆₀* reverts to lysine prototrophy predominantly after action by frameshift mutagens (personal communication from G. Mohn). *E. coli* WP2 *uvrA trp* is an *E. coli* B strain recommended by Green and Muriel¹¹ for base-change mutagen detection. The transferable drug resistance factor R46 has been shown to carry mutator genes that can enhance mutagenesis by both frameshift and base-change mutagens^{12,13}. R⁺ strains were constructed by conjugation using *E. coli* J6-2(R46) as a donor¹⁴.

All strains tested showed significant mutation in the fluctuation test after exposure to MMS. The *arg₅₆*, *gal R₁₈^s* and *trp* mutations all showed increased mutation frequency in the presence of 0.1% Mumbles mussel extract although as with the yeast experiments, in general, the non-liver fraction seemed to carry the major genetic activity. In contrast, the *E. coli* cultures carrying the auxotrophic marker *lys₆₀*, which carries a frameshift mutation, failed to show any increase in mutation frequency in the presence of

mussel extracts.

Table 3 shows the results of plate incorporation tests using both *E. coli* and *S. typhimurium*. Again the *E. coli arg₅₆* and *trp* loci showed significant reversion in R-factor-containing strains after exposure to mussel extracts. The *S. typhimurium his* mutants showed an increase in mutation frequency, induced by mussel extracts, in strain TA98, which produces prototrophic colonies by frameshift mutation, but not in strain TA100, which produces prototrophs by base substitution¹⁵. The failure of the latter strain to detect mutations induced by mussel extract reflects the specificity of the strains used.

Control experiments with both bacteria and yeast (briefly outlined in Tables 1 and 2), which will be described in further detail elsewhere, showed that the mussel alcohol extracts did not contain additional nutrient supplementation. Therefore the increases in prototrophs observed with the various strains were due to induction and not to a simple increase in cell division with a concomitant increase in spontaneous mutation. Further controls also showed that the mussel extracts were free of radioactive material.

Our results provide convincing evidence for the presence of mutagens that have become concentrated in the body tissues of mussels obtained from a number of maritime environments. The numerous observed correlations between chemicals showing convertogenic or mutagenic activity and carcinogenic activity¹⁵⁻¹⁷ suggest that such agents also represent a possible source of chemically induced carcinogenesis. At present our results do not provide direc

Table 3 Mutation induced in *E. coli* and *S. typhimurium* tester strains by mussel extracts as shown by plate incorporation tests

Strain	R factor	Mussel extract	Locus	Mean no. of prototrophs per plate
<i>E. coli</i> 343/113	—	Control	<i>arg</i> ₅₆	4.0 ± 1.0
		Mumbles (L)		9.0 ± 1.8
		Mumbles (NL)		9.9 ± 1.5
<i>E. coli</i> 343/113	+	Control	<i>arg</i> ₅₆	206.3 ± 11.3
		Mumbles (L)		671.0 ± 21.0
		Mumbles (NL)		Not done
<i>E. coli</i> WP2 <i>uvrA</i>	—	Control	<i>trp</i>	4.0 ± 1.0
		Mumbles (L)		6.3 ± 1.2
		Mumbles (NL)		7.0 ± 1.2
<i>E. coli</i> WP2 <i>uvrA</i>	+	Control	<i>trp</i>	28.3 ± 3.6
		Mumbles (L)		38.7 ± 6.2
		Mumbles (NL)		72.7 ± 3.5
<i>S. typhimurium</i> TA98	+	Control	<i>hisD</i> ₃₀₅₂	13.6 ± 1.6
		Plymouth (W)		104.5 ± 4.6
<i>S. typhimurium</i> TA100	+	Control	<i>hisG</i> ₄₆	61.5 ± 5.5
		Plymouth (W)		74.0 ± 6.1
<i>S. typhimurium</i> TA98	+	Control	<i>hisD</i> ₃₀₅₂	5.5 ± 1.0
		Mumbles		22.0 ± 2.1
<i>S. typhimurium</i> TA100	+	Control	<i>hisG</i> ₄₆	163.8 ± 5.7
		Mumbles		174.0 ± 5.9

The method used was essentially that of Ames *et al.*⁷. Overnight cultures of the tester strains were washed in saline and resuspended in saline. Washed bacterial culture (0.1 ml) plus 0.1 ml of mussel extract were added to semimolten minimal agar at 46 °C containing glucose and relevant supplements. These overlays were poured over minimal media plates containing glucose. The plates were normally scored after 3 d of incubation at 37 °C. W, whole mussel extract; L, liver; NL, non-liver.

evidence of the nature of the chemicals we detected. But the active extracts came from mussels collected in areas with obvious industrial pollution or in close proximity to industrial areas.

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Species richness and stability of space-limited communities

ECOLOGICAL competition theory has reached its fullest state of development for the special case of competition for a renewable resource^{1,2}. There are, however, many instances of competition in nature to which this theory does not apply; for example, competition for space (*Lebensraum*). Because there are many space-limited organisms in nature, including many primary producers, this is an important gap in our theoretical under-

standing of ecological communities. Moreover, since the fundamental dynamic of competition for space—spatial mobility—is quite different from that of competition for a renewable resource, it would be unwise to try to extrapolate the theory of resource limitation to space-limited communities. In this paper, I outline some calculations, in the spirit of refs 3–7, which bear on the relation between stability and diversity for space-limited communities. (Full details will appear elsewhere.)

It is now widely accepted that in general mathematical models of multispecies communities, complexity *per se* tends to beget instability rather than stability (ref. 2, ch. 3). There are also traces of this tendency in space-limited communities, but for a certain class of space-limited communities, complexity (in the sense of species richness) promotes (local) stability.

The model used is a system of reaction-diffusion equations^{6,8,9} of the form

$$\frac{dx_i^\mu}{dt} = \left[C_i x_i^\mu \left(k_i - \sum_j a_{ij} x_j^\mu \right) \right] + \left[D \sum_v (x_i^v - x_i^\mu) \right] \quad (1)$$

The meaning of this equation is as follows (for a more detailed description, see ref. 6). The habitat is partitioned into *m* patches of space and is populated by *n* species; x_i^μ , $\mu \in \{1, \dots, m\}$, $i \in \{1, \dots, n\}$, is the number (or biomass) density of species *i* in patch μ . Within each patch, the species compete among one another according to (for the sake of concreteness) a Lotka–Volterra model. This is expressed by the first square bracket in equation (1), in which k_i is the carrying capacity for species *i*, the a_{ij} are the competition coefficients, and $C_i k_i$ is the growth rate of species *i*. The competition coefficients a_{ij} are chosen so that the corresponding Lotka–Volterra model allows no stable equilibrium with any pair of species present (which seems the most characteristic sort of competition for space). Each species is, however, allowed to disperse randomly among the patches at a rate characterised by a constant *D*; this dispersion is such that there will be a net flow of a given species from patches with a relatively high density into patches with a relatively low density of that species. This is expressed by the second half of equation (1).

Consider colonisation of newly available space in such a model, taking the following initial conditions: the *n* species are randomly distributed among the *m* patches, with only 1 species per patch, and its density is in each case near its carrying capacity. Using the above equations the system will, for sufficiently small *D*, approach a stable equilibrium, with all species present in each patch⁶. I will call this a ‘colonisation equilibrium’ of the model.

The stability behaviour of an equilibrium can be characterised by means of the quantity $\Lambda = \text{minimum} \{ -\text{Re}(\lambda_A) \}$, where $\{\lambda_A: A = 1, \dots, mn\}$ are the eigenvalues of the linearised stability matrix of the system, evaluated at the equilibrium². ($\text{Re}(z)$ means the real part of *z*, for any complex *z*.) The condition for stability in the sense of Liapunov is $\Lambda > 0$ (which is just the familiar condition that all the eigenvalues have negative real parts), but the magnitude of Λ contains additional information: in case the equilibrium is Liapunov stable, then Λ^{-1} is of the order of the time scale for the exponential return of the system to equilibrium, if it has been moved away from equilibrium by some (small) disturbance. Alternatively, if the effects of random environmental fluctuations are represented by taking some parameter in the model to be a Gaussian random variable with variance σ^2 , then an approximate criterion for ‘stochastic stability’ of the equilibrium (meaning that the environmental fluctuations are unable to drive the system very far from equilibrium) is $\Lambda > \sigma^2$ (refs 2, 10). Thus, among Liapunov stable equilibria, Λ is a meaningful measure of the degree of stability: the larger Λ , the more stable the equilibrium.

To determine how the stability of colonisation equilibria of the above family of models depends on the number, *n*, of colonising species systems with different values of *n* are needed

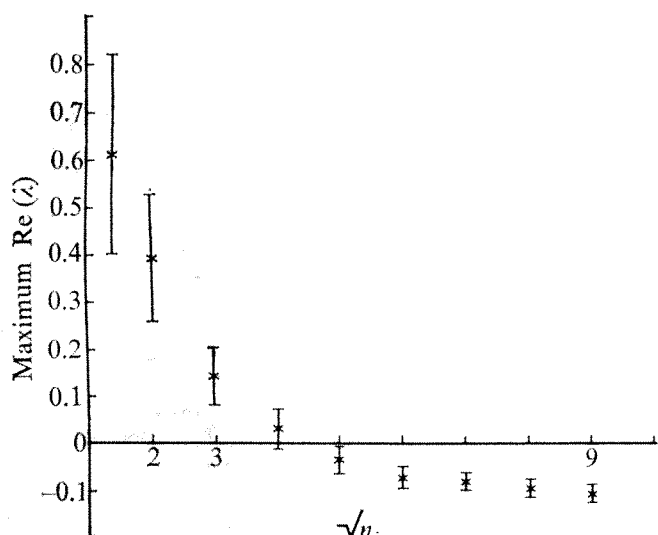


Fig. 1 The average (for the statistical ensemble defined in the text) largest real part of the stability matrix eigenvalues against \sqrt{n} , calculated by a numerical Monte Carlo method, for the parameter values $m = 1,000$, $D = 0.0002$, $(a, b) = (1.1, 1.9)$. The error bars are for one standard deviation. For systems chosen entirely at random, this quantity increases with n , rather than decreasing as here (ref. 2, Fig. 3.6).

which are, however, comparable in an appropriate sense. First, for a fixed habitat, the number, m , of patches is fixed throughout the following discussion. For each value of n , consider the statistical ensemble of systems having this number of species, for which the various parameters of the system are chosen at random from some fixed intervals of real numbers, and the n species are initially distributed at random among the m patches, with one species per patch. The average value of Λ is calculated for each n for the ensuing colonisation equilibria over this ensemble, and is $\langle \Lambda(n) \rangle$. The function $\langle \Lambda(n) \rangle$ of n indicates how, on average, the stability of such systems varies with n .

It transpires that there are two possibilities. Let (a, b) be the interval from which the competition coefficients α_{ij} ($i \neq j$) are chosen. Normalising α_{ii} to one for all i and scaling the species densities so that the carrying capacities k_i are all equal to unity, the condition for instability of all pairwise interactions (without dispersion) is $\alpha_{ij} > 1$ for all i, j with $i \neq j$. (This assumes no functional dominance, which will be discussed elsewhere in these pairwise interactions.) $1 < a < b$ must always be the case. The choices made for the other parameter intervals do not affect the qualitative nature of the outcome, which is as follows. If $a > 2$, then $\langle \Lambda(n) \rangle$ is a monotonically decreasing function of n , and if $(a+b)/2 < 2$, then $\langle \Lambda(n) \rangle$ is a monotonically increasing function of n .

Figure 1 shows an example of this latter behaviour (with $(a, b) = (1.1, 1.9)$). To facilitate comparison with earlier results² for stability matrices chosen completely at random, I have plotted the average largest real part of the stability matrix eigenvalues (that is, $-\langle \Lambda(n) \rangle$) against \sqrt{n} . The error bars are for one standard deviation. Figure 1 compares directly with Fig. 3.6 of ref. 2: the numbers depicted were obtained by a Monte Carlo method.

So, for $a > 2$ (which means roughly that the competition is constrained to be sufficiently "strong") increased species richness tends to destabilise the colonisation equilibria, whereas for $(a+b)/2 < 2$ ("weak" competition) increased species richness tends to stabilise the colonisation equilibria. The implications of this for the overall stability of the system depends on the global structure of each model. For some models, stochastic instability of the colonisation equilibria, in the sense that the variance σ^2 of environmental fluctuations exceeds Λ , will mean that the spatial structure of the system will be fluctuating constantly from one pattern to another, but with all n species present (compare ref. 7); whereas for other models it will mean

a high probability that one or more species will become extinct, on an ecological time scale.

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Effects of drought on grain growth

DROUGHT severely decreases grain yield in many cereal growing regions and the resulting fluctuations in world food supply have serious repercussions¹. Crop breeders and physiologists are therefore studying varietal characteristics that may enable cereals to yield more consistently in drought conditions². Very few precise field measurements show how drought affects grain growth, but procedures have been advocated to provide a more precise description of grain growth and a more rigorous examination of sources of assimilate used for grain growth³⁻⁵. We have used these procedures to analyse how the droughts of 1975 and 1976 affected grain growth of wheat crops in England and to define particular physiological traits which govern yield.

The analysis is based on detailed measurements of crop growth made on three crops of Maris Huntsman winter wheat, grown in the Midlands on the same soil type in 3 consecutive years. The crops were grown according to normal agronomic practice and were chemically protected against diseases, pests and weeds. Actual evaporation (E_a) was measured using a neutron probe technique⁶ and potential evaporation (E_p) was estimated from a standard formula⁷. Table 1 gives the grain yields of the three crops and pertinent weather observations: 1976 was the driest growing season at Sutton Bonington since records began in 1916. E_p and temperature during grain growth increased markedly from 1974 to 1976, whereas E_a decreased.

For analysis, grain growth rate can be assumed constant^{3,8,9} and final mean weight per grain ($\bar{W}_g(f)$) can be expressed as:

$$\bar{W}_g(f) = \bar{W}_g(i) + (R_g \times D_g) \quad (1)$$

where $\bar{W}_g(i)$ is the initial value of \bar{W}_g , R_g is the grain growth rate during the linear phase of growth and D_g is the duration of that phase³. Increase in \bar{W}_g was assumed to be linear once \bar{W}_g exceeded 6 mg, and Fig. 1 shows the grain growth pattern

Fig. 1 Increase in mean weight per grain with time in 1974 (\square), 1975 (\triangle) and 1976 (\circ).

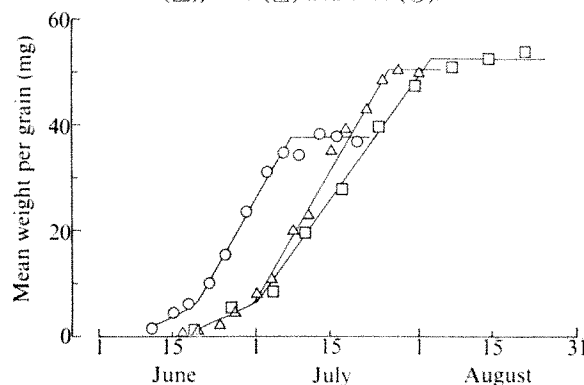


Table 1 Grain yield, weather and grain growth observations for the three wheat crops

Year	Dry matter grain yield* (g m ⁻²)	Duration (d)	Anthesis until cessation of grain growth		E_a/E_p	During linear grain growth				Final mean weight per grain (mg)
			Actual evaporation [E_a] (mm d ⁻¹)	Potential evaporation [E_p] (mm d ⁻¹)		Mean air temperature (°C)	Mean maximum air temperature (°C)	Mean grain growth rate [R_g] (mg d ⁻¹)	Grain growth duration [D_g] (d)	
1974	495 (46)	45	3.0	3.0	1.00	14.9	19.5	1.41	32.9	52.2
1975	535 (51)	38	2.0	3.4	0.59	16.8	21.6	1.76	25.2	50.3
1976	340 (32)	25	2.1	4.6	0.46	20.7	28.5	2.11	14.5	36.6

*Farm yields in cwt acre⁻¹ at 15% moisture are shown in parentheses.

for the 3 yr. Table 1 gives the relevant values of the variables of equation (1). Compared with 1974 the warmer, drier weather of 1975 was associated with a slightly faster R_g but this was offset by a decrease in D_g so that $\bar{W}_g(f)$ was close to 50 mg in both seasons, a typical figure for Maris Huntsman¹⁰. In 1976 very dry weather and exceptionally high temperatures were associated with very fast R_g (Table 1). But this was more than offset by a sharp decrease in D_g so that $\bar{W}_g(f)$ was only 37 mg.

Changes of plant dry weight after anthesis must be examined in greater detail to establish the reason for the extremely short D_g in 1976.

It is convenient to distinguish two sources of assimilate supply for grain growth: photosynthesis after anthesis, and translocation of materials assimilated before anthesis and temporarily stored before moving to the grain. As a first step in assessing the importance of these two sources, increase in grain weight from anthesis to final harvest (ΔW_g) can be expressed as

$$\Delta W_g = \Delta W_t - \Delta W_s$$

where ΔW_t is the increase in total crop weight, and ΔW_s is the increase in weight of plant parts other than the grain between anthesis and harvest⁵. By far the largest proportion of ΔW_s consists of the stem and for brevity this term is referred to as stem weight. In Table 2, which shows the relevant crop dry weight changes, $-\Delta W_s/\Delta W_g$ is the fraction of final grain weight apparently derived from translocation of materials assimilated before anthesis. Even in 1974, this fraction (0.35) was much larger than the maximum of about 0.20 expected for wheat¹¹. In 1975 and 1976, however, when production of dry matter after anthesis was smaller still in relation to the requirements for grain growth, the contribution of translocated materials to grain yield was much greater. This increased movement of materials from stem to grain when conditions during grain filling are adverse for photosynthesis lends support to the concept of "compensatory translocation"⁵. The small value of $\bar{W}_g(f)$ which occurred in 1976 in spite of considerable translocation of material from the stem raises two questions: is there a limit to the amount of dry matter that can be supplied to the grain by compensatory translocation; was this limit reached or exceeded in 1976?

The extent of translocation may ultimately be limited by the need to retain sufficient dry matter in the stems to support the ears. In 1976, however, supporting tissue per grain was considerably greater than in 1975 (Table 2). Thus it seems that shortage of assimilate did not limit final grain weight by curtailing the duration of grain growth. There are two other possible reasons why grain growth stopped: either the transport

system to the growing grain was inadequate or starch synthesis stopped prematurely. Studies of translocation during wheat grain growth show little effect of water stress¹², but there is evidence that a fall in the synthetic capacity of the endosperm causes grain growth to stop¹³. In 1975 and 1976, the duration of starch synthesis could have been limited either by the effect of drought on plant water status or by abnormally high ear temperatures. The relative importance of these two correlated factors cannot be determined from our measurements, but related work in controlled environments indicates that temperature rather than water stress stops grain growth prematurely¹⁴⁻¹⁶.

These results illustrate the potential importance of translocation of material assimilated before anthesis to grain growth when current photosynthesis is limited by an adverse environment. They also establish the usefulness of treating grain growth as a linear process and expressing final weight per grain as a product of a growth rate and duration. If cereals are to yield well in regions where drought and high temperatures during grain filling are frequent, varieties must be bred which not only increase the rate but also sustain the duration of grain growth. Such characteristics would maximise the assimilate to be harvested in the nutritionally valuable fraction of the crop.

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Table 2 Crop weight measurements

Year	Increase in weight (gm ⁻²) between anthesis and harvest				Stem weight per grain (mg)
	Grain [ΔW_g]	Total [ΔW_t]	Stem [ΔW_s]	$-\Delta W_s/\Delta W_g$	
1974	495	322	-173	0.35	65
1975	532	228	-304	0.57	54
1976	341	149	-192	0.56	63

Predicting the course of Gompertzian growth

THE increase in volume or size with time that characterises many biological and physical systems is often well approximated retrospectively by mathematical 'growth curves'. In some cases, however, growth may be sufficiently complicated for it to be impossible to predict later portions of the growth curve if observations are limited to a few early points. We report here the development of a generalised approach to

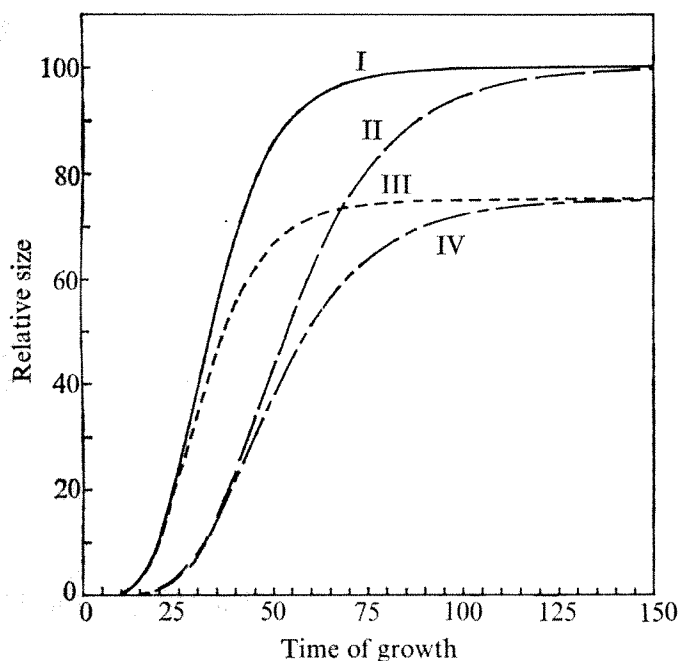


Fig. 1 Hypothetical Gompertzian curves derived by solving equation (3) with various parameters A and B as follows: Curve I, $A = 0.4$, $B = 0.4/(\ln 100)$; Curve II, $A = 0.25$, $B = 0.25/(\ln 100)$; Curve III, $A = 0.4$, $B = 0.4/(\ln 75)$; Curve IV, $A = 0.25$, $B = 0.25/(\ln 75)$. Note that the early portion of the curve reflects parameter A and the later portion of the curve reflects the ratio of A/B .

the analysis of "Gompertzian" growth which enables accurate predictions of future growth for two model tumour systems. This mathematical method may be useful clinically, and expresses a property of biological growth that may be applicable to other systems.

The growth of most experimental neoplasms²⁻⁴, human populations⁵, normal embryos⁶, molluscs⁷, visceral organs^{8,9} and some human tumours¹⁰ are well described by the S-shaped curves used for actuarial purposes by Benjamin Gompertz in 1825 (ref. 1). The essential distinguishing feature of Gompertzian growth (Fig. 1), is some mechanism of feedback inhibition with increasing size, resulting in decremented exponential growth that achieves a limiting size asymptotically. Unfortunately, the relatively complicated form of the Gompertzian equation has tended to limit its general applicability, often resulting in the alternative use of less satisfactory exponential¹¹⁻¹⁴, summed exponential¹⁵, or other¹⁶ growth models. These other models however, are rarely applicable to an entire growth history, and are therefore insufficient for the purposes of predicting later growth.

A Gompertzian function is the simultaneous solution of the two differential equations

$$dN(t)/dt = K_1 \cdot N(t) \cdot G(t) \quad (1)$$

$$dG(t)/dt = -K_2 \cdot G(t) \quad (2)$$

where K_i is a constant > 0 , $N(t)$ is volume at time t , and $G(t)$ is a function entirely described by equation (2). The expression $K_2 \cdot G(t)$ can be thought of as the fraction of $N(t)$ that doubles in size during the instant dt . The solution of these equations is often written in the form

$$N(t) = N(0) \cdot \exp[(K_1/K_2) \cdot G(0) \cdot \{1 - \exp(-K_2 \cdot t)\}]$$

Since

$$G(t) = G(0) \cdot \exp(-K_2 \cdot t) = (K_2/K_1) \cdot \ln[N(\infty)/N(t)]$$

where $N(\infty) = \lim_{t \rightarrow \infty} N(t)$, an alternative form of equation (1) is

$$\begin{aligned} dN(t)/dt &= K_2 \cdot N(t) \cdot \ln[N(\infty)/N(t)] \\ &= A \cdot N(t) - B \cdot N(t) \cdot \ln[N(t)] \end{aligned} \quad (3)$$

where $B = K_2$ and $A = K_2 \cdot \ln[N(\infty)]$. The dependence of the pattern of growth of $N(t)$ on A and B is illustrated in Fig. 1. For a given $N(0)$, when A is constant, the early phase of growth (often referred to loosely as the exponential phase) is similar in spite of different values of B . Also, when A/B is constant, the later phase of growth (the plateau phase) is similar in spite of different values of A . Were A and B unrelated, knowledge of the early phase of growth of a particular system (tumour, organ, embryo, or population) would not enable an accurate prediction of the later phase, as the value of A , but not A/B , could be estimated from the early growth pattern. Similarly, measurements of late growth would provide an estimate of A/B but not A . On the other hand, if it were possible to estimate B from A , the entire growth pattern of an individual system would be predictable on the basis of measurements early in its growth history.

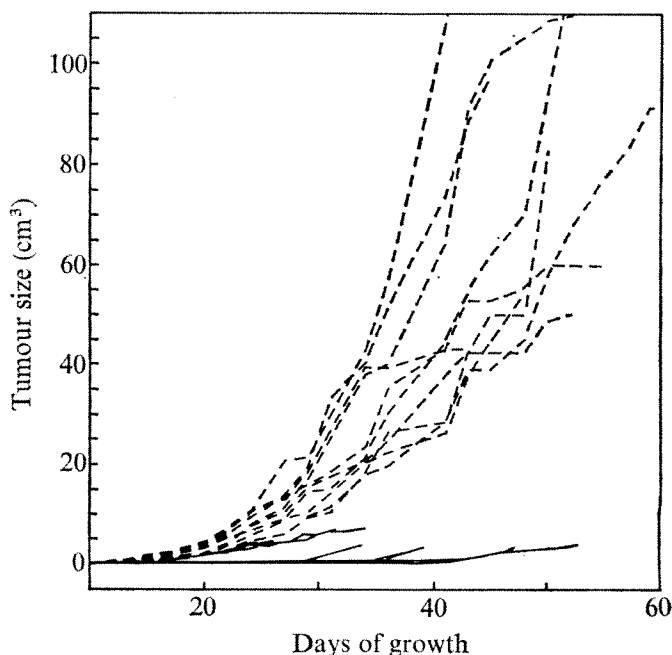
For eight cases of B-16 melanoma in BDF₁ mice and ten cases of transplantable mammary carcinoma 13672 in Fischer 344 rats, measurements of length (L) and width (W) were obtained during unperturbed growth from the time of transplant until the death of the host or the occurrence of tumour ulceration. Approximate tumour volumes (V) were calculated as the volume of revolution of an ellipse by the formula

$$V = (\pi/6) L W^2$$

The growth of these tumours (Fig. 2) demonstrates the variability typical of malignant proliferation.

For each tumour, the solution of equation (3) was fit using the method of least squares to the curve of volume as a function of time of growth starting from the hypothetical volume of a single cell. Examples of curves fitted to data for each tumour type are illustrated in Fig. 3. Each curve fit yields the two parameters, A and B , that completely describe the growth pattern

Fig. 2 The volume of eight examples of B-16 melanoma (—) and ten examples of 13672 carcinoma (---) plotted as a function of time from initiation of growth. The volume scale is arithmetic. The variability in growth pattern typical of neoplastic proliferation is evident.



for that tumour. As seen in Fig. 4, when $\exp(A)$ is plotted against $\ln(B)$ for all individuals of each tumour type, the relationship is found to be linear with correlation coefficients of 0.99 for B-16 melanoma ($P < 0.0000002$) and 0.96 for the rat carcinoma ($P < 0.000006$). This high degree of correlation between the transforms of A and B allows, for these experimental tumours, the accurate prediction of B on the basis of an estimate of A made from measurement of the early phase of growth of an individual tumour. Equation (3) may therefore be written

$$dN(t)/dt = A \cdot N(t) - \exp[(\exp\{A\} - n)/m] \cdot N(t) \cdot \ln[N(t)] \quad (4)$$

a Gompertzian equation in one variable, A , with constants n , m , and $N(0)$ (the volume of a single cell) specific for a particular tumour type.

Having determined empirically the constants n and m for a tumour type, the total growth curve for an individual tumour of that type can be predicted accurately from a few early measurements of volume. In Fig. 3, for example, the solution of equation (4) was fit to three early measurements of a tumour not used to derive the above relationship between A and B . The prediction obtained is seen to match closely the actual volumes observed later in the growth of the tumour.

The ability to predict the later growth pattern of an individual tumour on the basis of early growth measurements should have widespread applicability, not only in the design of experimental anti-cancer therapy, but in the diagnosis and prognosis of human tumours as well. For example, the response of an individual cancer to treatment could be expressed in terms of deviation from ideal unperturbed growth as predicted from measurements taken before therapy. This precise method of monitoring therapy has obvious advantages over parameters presently in use, such as rate of complete or partial remission or average tumour size at some arbitrary point after therapy. These conventional endpoints are inapplicable to individual

Fig. 3 Examples of equation (3) fit to data for B-16 melanoma (\square) and 13762 rat carcinoma (\triangle). The volume scale is logarithmic. The dashed lines represent the fitted curves. Paired constants (A , B) derived from several such curve fits are plotted in Fig. 4. The points marked 0 are three early volume measurements for an individual 13762 rat carcinoma not used to derive the constants n and m (see text). Equation (4) was fit to these three points using constants n and m from Fig. 4; the resulting solid line is seen to predict accurately the later growth measurements (\bullet) for that tumour; measurements unknown at the time of curve fitting.

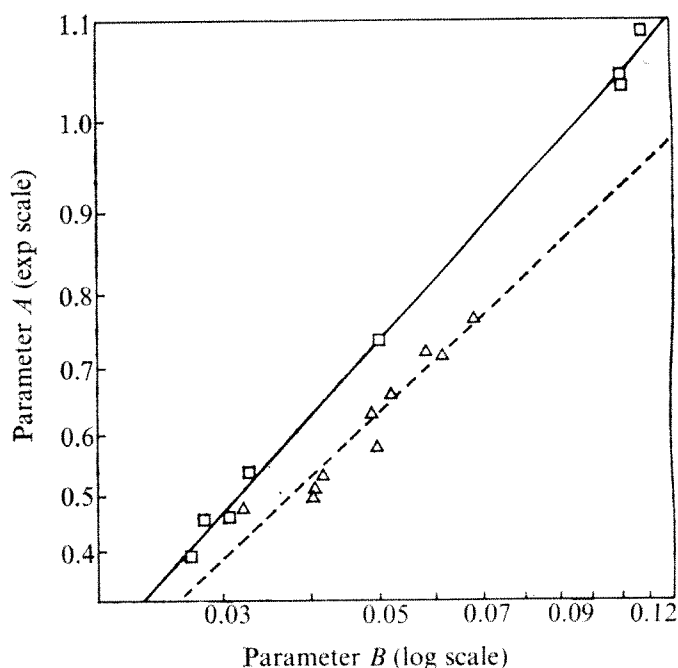
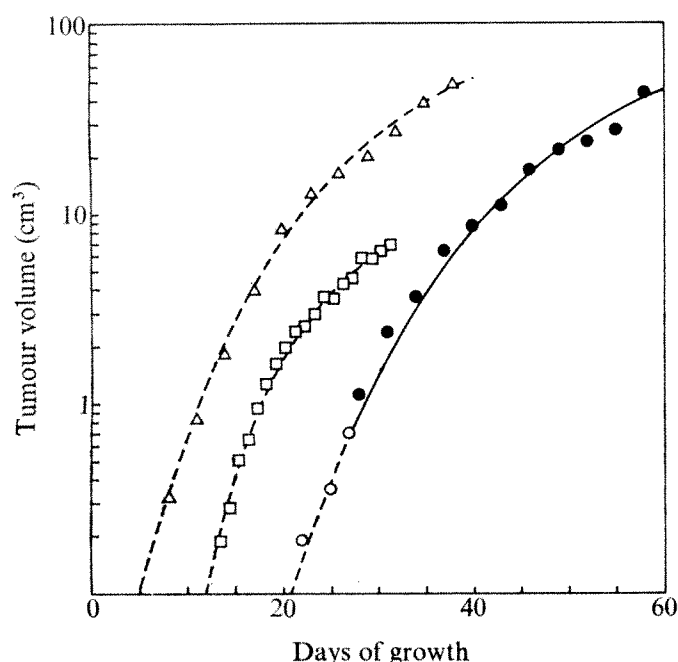


Fig. 4 Correlation between $\exp(A)$ and $\ln(B)$ for B-16 melanoma (\square) and 13762 rat carcinoma (\triangle). The points represent pairs of parameters (A , B) determined from curve fits as illustrated in Fig. 3; the solid lines represent the regression equation $\exp(A) = m \cdot \ln(B) + n$, with the following values of m , n , respectively: B-16 melanoma, 0.9645 and 4.980; 13762 carcinoma, 0.8176 and 4.334.

tumours, are potentially biased by drug or other non-tumour deaths, and may underestimate the influence of weakly effective forms of therapy which may nevertheless be potentially useful in combinations.

For these two experimental tumours the methods described here reveal a remarkable orderliness of growth that tends to dispel the concept of cancer as a wild and uncontrolled phenomenon. We may expect that the growth of non-neoplastic tissues (for example, gut epithelium, bone marrow and regenerating liver) would be at least as orderly as that of these two malignancies. Results obtained by applying these methods to normal tissues will be reported later. These mathematical methods may also be applied to the growth of mammalian or bacterial cells in culture, with obvious use in the monitoring of growth inhibitory influences (such as hormones and antibiotics). We also plan to describe the applications of these methods to other tumour types, paying particular attention to the description and prediction of the response of malignancies to single and multiple agent therapies.

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Significance of cell shape in tissue architecture

STIMULI passing to a cell within solid tissue are modulated by the cellular environment. Control mechanisms must therefore involve both humoral influences and the architecture of the cellular microenvironment. Epidermis suffers a constant loss of surface cells which are replaced by basal cells which have differentiated during migration to the surface. In mouse ear and dorsum, the path of this migration can be observed as precisely defined columns of cells¹ which are in tetrakaidecahedral² form (Fig. 1) as in the rigid cell walls of plant tissues³. Recent studies^{3,4} have suggested that maintenance of this system of 'dynamic orderedness' may be explained by reference to its physical geometry, and minimal surface packing configurations. In this paper we discuss some of the consequences of this view of tissue architecture.

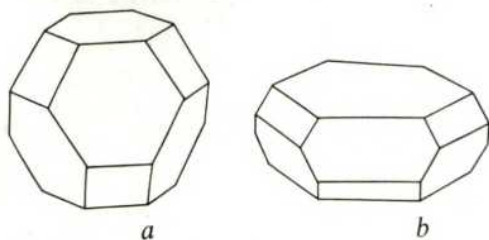


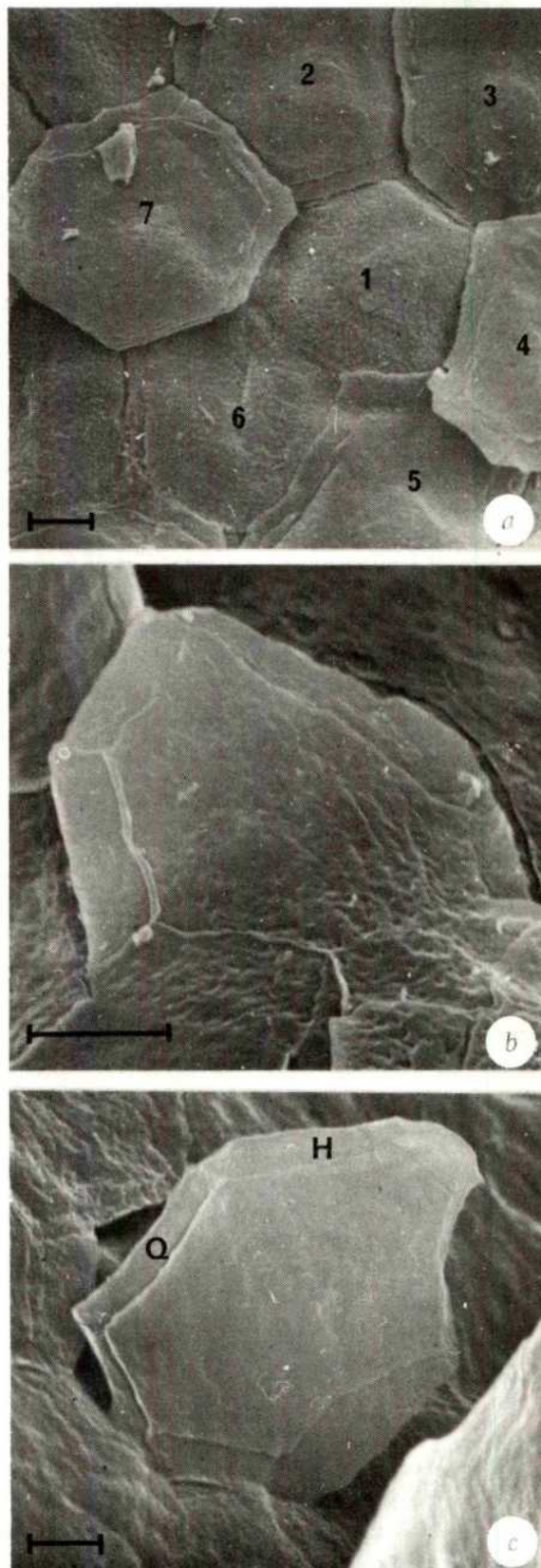
Fig. 1 a, Line drawing of an orthic tetrakaidecahedron. This 14-sided figure consists of six equal opposite quadrilateral faces and eight equal and opposite hexagons. Each quadrilateral face is bounded by four hexagons and each hexagonal face by three hexagons and three quadrilaterals. **b**, When compressed about a pair of hexagonal faces the shape assumed will become predominantly hexagonal, with the remaining 12 faces becoming hexagonal and quadrilateral facets around the edges.

The ordered epidermis of mouse ear or dorsum shows keratinised squames piled in columns and presenting a characteristic hexagonal patterning^{1,5}, when viewed from the surface (Fig. 2). It is clear from the flattened nature of these cells that if they are tetrakaidecahedral, then the orthic form of the tetrakaidecahedron has become distorted to a flattened version (Fig. 1), in which the flattening has resulted in an increase in area of a pair of the hexagonal faces, reducing the remaining 12 faces to alternate quadrilateral and hexagonal facets around the edges.

Fig. 2 a, Area of mouse ear epidermis showing a central hexagonal squame (1) and its six surrounding neighbours (2-7). Squame 1 is overlapped on all edges, and so cannot desquamate until all the surrounding cells (2-7) have done so, whereas squame 7 has all edges free and is able to desquamate. The region of overlap (previously covered by the last squame in the central pile) and the interdigitating facets are visible at the edges of the surrounding squames. At this advanced stage of flattening that occurs close to the surface, the facets at the squame edge have largely lost their characteristic alternate hexagonal and quadrilateral appearance because of the overall distortion, but still provide evidence of the tetrakaidecahedral form adopted in the less keratinised and more plastic regions of the granular layer. Scale bar: 5 μ M. **b, c**, Cells exposed from some distance below the surface showing clearly the facets at the edge providing the interdigitating regions. In some cells (**c**) a close approximation to the alternate hexagon (H) and quadrilateral (Q) faceting seen in the true geometric tetrakaidecahedron (Fig. 1b) is apparent. Scale bars: 5 μ M.

These facets may be observed in the scanning electron microscope particularly if some of the outer squames are removed (Fig. 2).

From the density packing configuration of either orthic or flattened tetrakaidecahedra, there is only one way in which these shapes can be assembled. This results in a columnar organisation in which the top 'cell' of the central column, of any group selected, occupies a position at the lowest or highest level, relative to the surrounding six adjacent columns which are



themselves subdivided alternately into two sets of three, each set at a different level (Fig. 3) rather than any of the other possible arrangements for hexagonal grid systems^{7,8}. Any individual surface cell may thus be considered to be surrounded by two sets of three, and as the individual itself is also part of a set of three, an entire area is thus split into sets of 'threes'. This approximates the observed structure of mammalian epidermis^{1,3,5,6} and was also suggested from a two-dimensional hexagonal grid system⁷ after consideration of control mechanisms.

Epidermal columns can be defined down to the spinous layer (Fig. 4) (that is the layer immediately above the basal layer) and each column must be maintained by vertical migration (and its associated cell division) in the group of 10 basal cells directly beneath the column as defined by the epidermal proliferative unit (EPU) concept^{5,6}. The cell kinetics of the epidermis⁹ suggest an entry of one cell per day into each column, which must equal the cell loss rate. Cell loss in a tetrakaidecahedral system is limited to individual cells, as each cell only becomes free to desquamate when it is slightly 'proud' of its six neighbours and has all its edges free (position 7 in Fig. 2a). Such an ordered desquamation in relation to tetrakaidecahedral cell shape implies a regular alternating overlap between neighbouring columns, as observed in epidermal sections^{6,7,10}. Communication between neighbouring columns seems necessary to ensure the correct sequence of cell migration⁷. Considered in profile alone (Fig. 4), for the regular alternating overlap of squame edges to be maintained, a column cannot introduce another differentiated cell into its squame pile until both neighbours have added to theirs. Extension of this concept to three dimensions involves consideration of the under surface of a group of seven tetrakaidecahedral columns (Fig. 3, inverted) and shows that the central column must be added to before those at the periphery. The overlaps of the peripheral columns may form a vertical channel to aid this sequence, and thus provide a partial self-assembly system which predetermines the column entered by the next migrating basal cell. This determination of the eventual position of each vertical migration imposes a sequence for cell migration and production on the basal layer. As there is a group of approximately ten basal cells positioned beneath each column (Fig. 4), it seems reasonable to assume that they provide cells directly into the column, as suggested by the EPU concept⁵: migration into a column from outside this group would be unnecessary and

Fig. 3 Drawing of a model of flattened tetrakaidecahedra assembled without interstices showing how the columnar arrangement develops. Three different levels are apparent in the group which consists of a central column (upper surface of the top cell black) and its surrounding six neighbours. The central column here is in the lowest position (black), the three columns arrowed right in the intermediate position, and the three arrowed left in the uppermost position. Thus when the diagram is inverted, the column ready to receive the next migrating basal cell will be the central column, and the raised edges of the six surrounding columns may provide a physical guide to facilitate such a migration.

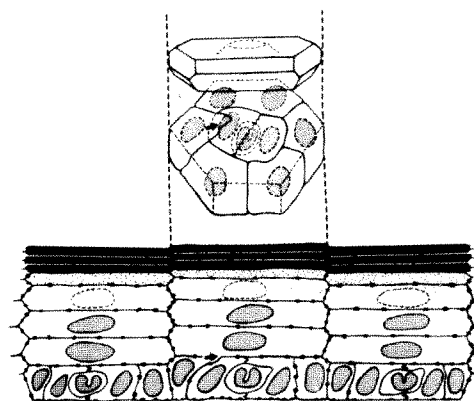
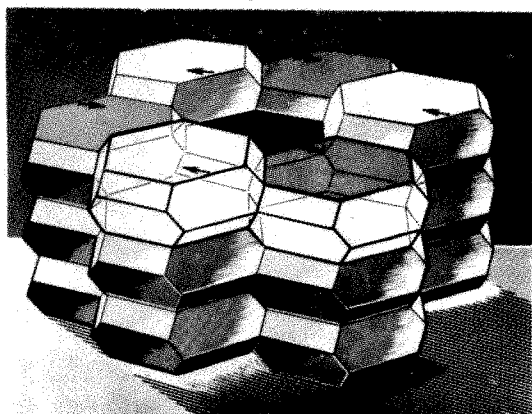


Fig. 4 Diagrammatic representation of three adjacent columns and their underlying basal cells as seen in profile. Here the peripheral columns have been the most recent to add to their stack of differentiating cells so will not add further cells until the central column has added a cell, otherwise the exact overlapping of squame edges would not be maintained. No column or EPU is independent of its neighbours as regards the vertical migration of basal cells into the spinous layer.

inefficient, involving extensive lateral migration of basal cells. A problem thus arises with the direct comparison made by Menton^{3,4} between cell columns in epidermis and columns of soap bubbles. The epidermis is a compressed series of cell layers without intercellular spaces, tightly bound together by desmosomes. Migration from the basal layer involves a major change of cell shape and extensive breaking/remaking of the desmosomal contacts. In a bubble tank, however, there is free space for lateral movement, and no bubble-to-bubble connections, so any bubble may jostle its way to the correct position in a column base regardless of its point of origin. The basic difference is, therefore, that soap bubble columns exist as passively formed stable arrangements of randomly generated bubbles, in free space, whereas skin columns are produced by what can only be an active control of cellular dynamics.

Although the bubble analogy is useful in understanding the precise geometry of the system, it seems unlikely that living cells become formed into columns because they have no option other than to pack in a minimum surface configuration. It is more likely that selection in epidermis has produced the most efficient system of barrier production, which in stacked areas of epidermis assumes a minimum surface configuration. We may also mention at this point that in plantar mouse epidermis, there is no apparent order and also that the orderedness in stacked epidermis breaks down in response to wounding. In stacked epidermis it therefore seems reasonable to view each column as an individually controlled entity, with cell replacement occurring in the group of basal cells directly beneath the column, that is, an epidermal proliferative unit^{5,6}. Two levels of control of the EPUs would be necessary to maintain columnar organisation: an intra-EPU control of cell division as a replacement process subsequent to migration, and an inter-EPU control providing a synchrony of vertical migration in trios over the epidermal surface. It seems clear therefore that the ordered structure of columnar epidermis itself precludes random basal cell migration and mitosis, and suggests perhaps that other cell renewal systems with slightly less obvious cellular architecture may maintain themselves by similar systems.

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Transformation of tissue-cultured xeroderma pigmentosum fibroblasts by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine

In vitro transformation by chemical carcinogens has been shown and established mainly in rodent cells¹. Attempts made to transform tissue-cultured human cells have so far failed². Only two seemingly promising reports on *in vitro* transformation of human cultured cells were those reported by Benedict *et al.*³ and Rhim *et al.*⁴. But the cell line used by Rhim *et al.* to transform by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) was derived from osteosarcoma and had itself many cytological as well as chromosomal features characteristic of malignant tumour cells. As we cannot imagine why human cells should not be transformed by the chemicals which are potent carcinogens for cells of rodent origin, we believe that successful transformation depends only on finding the optimal experimental conditions and suitable cells.

Xeroderma pigmentosum (Xp) is a recessive hereditary disease characterised by a marked sensitivity to sunlight (ultraviolet irradiation) which often results in formation of skin tumours. This disease is due to a genetic defect of the excision repair system^{5,6}, analogous to the *uvr* mutations in *Escherichia coli*. We have been interested in this disease because it may help to clarify the mechanism of carcinogenesis by allowing the study of the relationship between the excision repair defect and malignant tumour formation. Here we report our preliminary results on an apparent transformation of fibroblasts derived from the skin of two Xp patients by treatment with MNNG.

A human Xp cell line designated CRL1200 was obtained from the American Type Culture Collection and used at 11th subculture level. This cell line was derived from an 11-yr-old girl with neurological complications and skin tumours in addition to ordinary skin symptoms characteristic of Xp; it had a normal fibroblast morphology. Another Xp cell line designated Xp-T was our isolate from a healthy part of skin derived from a 32-yr-old Japanese male with lip cancer in addition to ordinary clinical symptoms of Xp; it was used at its 9th subculture level. As a normal control a fibroblast culture derived from a 26-yr-old Japanese male without any positive reason to suspect genetic abnormalities was used at its 10th subculture level and was designated as Non-Xp.

Growth and maintenance medium consisted of Eagle's minimal essential medium (MEM) with 10% foetal bovine serum, 100 units of penicillin and 50 µg kanamycin ml⁻¹.

One day before MNNG treatment, cells were trypsinised at their confluent monolayer stage in Falcon 250-ml plastic flasks and 20% of them were discarded, to obtain cells growing actively at the time of MNNG treatment. After growth for 24 h in an atmosphere of 5% CO₂ in air at 37 °C, cells were again trypsinised and resuspended into the original volume of medium containing MNNG at 1 × 10⁻⁵ M. MNNG was dissolved in a minimal amount of dimethyl sulphoxide and then growth medium was added to give the concentration which was equivalent to the 10% killing dose as judged by the nigrosin vital staining against Non-Xp cells under the conditions employed for MNNG treatment. After 60 min treatment with MNNG in a CO₂ incubator at 37 °C, cells were centrifuged at 750 r.p.m. for 10 min, washed with

fresh medium and then inoculated at 1 × 10⁶ cells per 15 ml medium per flask. The treated cultures were then grown in a CO₂ incubator with medium change twice a week. After 26 d incubation, cells were trypsinised to respread in the same flask, and further grown with a similar schedule of medium change. On the 32nd and 39th days, one flask of the cultures was subcultured into two flasks.

On the 43rd day the MNNG-treated CRL1200 culture produced several focus-like spots which still consisted of spindle-shaped cells, although their arrangement was altered and criss-crossed. The MNNG-treated Non-Xp cells showed a similar morphological alteration up to this stage but reverted to the normal arrangement soon after. The arrangement of some of these focus-like spots in the MNNG-treated CRL1200 culture only changed progressively and randomly oriented multilayers began to form around the centres of the spots on about the 63rd day. But constituent cells still had a spindle-shaped fibroblast appearance. On the 77th day multilayer formation at the foci became more and more marked until under a microscope cells on the top of each focus could no longer be observed simultaneously with the cells around the focus directly attached to the flask surface. Cells on the top of foci lost the fibroblastic appearance and were converted into small epitheloid cells with only one to two nucleoli, round or polygonal cytoplasm and an ovoid or round nucleus. The peripheral area around these foci was also altered to consist of a criss-crossed cell arrangement where giant cells supposedly formed by nuclear fusion were often observed, and made a marked contrast to the unaffected outer area which consisted of fibroblasts with monolayer orientation.

The multilayered foci were formed only in the MNNG-treated CRL1200 culture but never in the untreated CRL1200 nor in the MNNG-treated Non-Xp culture. These cultures were trypsinised on the 85th day and chromosome counts were made. Only the MNNG-treated CRL1200 culture with marked multilayered foci showed polyploidy and heteroploidy although the majority of cells in these cultures remained unaffected and grew normally as spindle-shaped cells. No marked change in chromosome counts was observed in the untreated CRL1200 culture.

On the 105th day after the MNNG treatment, cultures were trypsinised and distributed into several flasks at a cell concentration of 1 × 10⁴ per flask. Then the increase in cell number and the percentage of cells in the S phase (as demonstrated autoradiographically by daily incorporation of ³H-thymidine (0.2 µCi ml⁻¹) following 24-h pulse labelling) were examined. As shown in Fig. 1, the MNNG-treated CRL1200 culture showed a higher cell saturation density than did the MNNG-treated Non-Xp culture. DNA synthesis in the MNNG-treated CRL1200 culture was observed

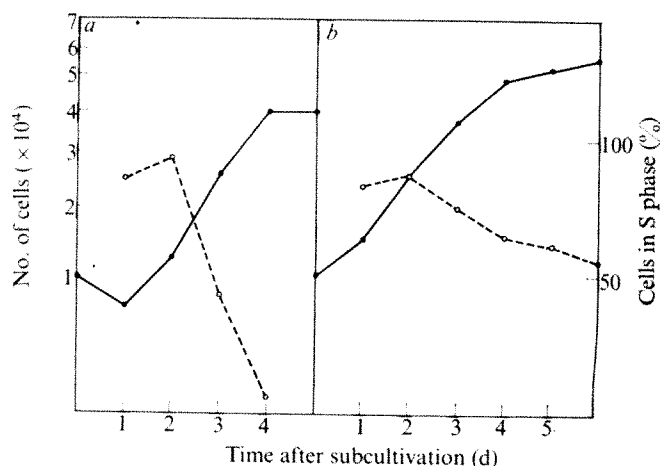


Fig. 1 Increase in cell number (solid line) and percentage of cells in the S phase (dotted line). a, Non-Xp; b, CRL1200 (Xp).

in 55% of the cells at saturation cell density but it was only 5% in the MNNG-treated Non-Xp culture. These observations suggested that the "transformed" CRL1200 culture, although still containing a large number of unaffected cells, consisted of cells showing "contact inhibition" less markedly than similarly treated Non-Xp cells. This was also confirmed in an autoradiogram which demonstrated that many of the cells constituting multilayered foci were in the S phase and that even the peripheral cells with monolayer orientation and fibroblastic appearance were actively synthesising DNA whereas unaffected cells incorporated almost no ^3H -thymidine label. Similar results to those described above for CRL1200 were obtained in another Xp strain, Xp-T.

These MNNG-treated transformed cells were examined three times for malignancy by transplantation into athymic nude mice which resulted in regression. Although tumorous growth was initially observed, histologically no sarcomatous pattern was shown. If the above described alteration in Xp cells after treatment with MNNG can really be considered as transformation, then it follows that only the Xp cells can be transformed by treatment with MNNG under the conditions employed but Non-Xp cannot. It had already been reported⁷ that cells from an Xp patient showed a higher frequency of transformation by SV40 than cells from the mother of the patient heterologous for the Xp gene. These observations suggest the interesting possibility of a common biochemical step between viral and chemical carcinogenesis and that this step itself is somehow affected in Xp patients.

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Distribution and valency of receptor for IgE on rodent mast cells and related tumour cells

MONOMERIC IgE binds tightly to the surface of mast cells and basophils without, by itself, causing any known perturbation. Interaction of the IgE with an appropriate antigen (or experimentally with anti-IgE) causes the cells to degranulate. Little is known about the initial molecular events in IgE-mediated triggering, but there is considerable evidence that crosslinking of the IgE molecules is a critical step (see refs 1 and 2 for discussion). The available data suggest that only limited bridging rather than an aggregation-induced extensive redistribution of the surface IgE^{3,4} is required; indeed if the bridging is extensive enough to induce such gross redistribution within the time that degranulation would ordinarily be expected, the latter is inhibited^{3,5}. Since bridging of the IgE, and therefore of the receptor to which it is bound, seems to be a critical signal, it is important to know if the cellular binding sites for IgE are integrated into larger functional units; the receptor molecules themselves

might be multivalent, or individual receptors might be connected to each other by some other component (Fig. 1). The experiments described here were directed towards investigating this point. We saturated cells with a mixture of two distinguishable types of rat IgE and determined whether by redistributing one of them with specific antibody, the other would comigrate.

Rat IgE was purified⁶ from the ascitic fluid of rats bearing the IgE-producing immunocytoma IR162 (ref. 7). Fluorescein-labelled IgE (F-IgE) was prepared by reacting 10 mg ml⁻¹ of IgE with 2 mg ml⁻¹ fluorescein isothiocyanate, adsorbed on celite⁸ at pH 9.6 for several days. After centrifugation the solution was passed over Sephadex G-25. The final preparation which was fully active (as determined by comparing it with native IgE for its capacity to inhibit the binding of ^{125}I -IgE) had an average of 12 mol fluorescein per mol protein. Rhodamine-conjugated IgE (R-IgE) was prepared in an analogous manner (by Dr J. Schlessinger) and was similarly fully active.

The antisera used were as follows: rabbit anti-rat IgE was prepared as described previously⁹; anti-fluorescein antibodies were prepared from horse anti-fluorescein serum¹⁰ (the gift of Dr D. W. Scott) by adsorption on a column of fluoresceinated bovine IgG-Sepharose 2B and, after washing, elution with 0.1 M acetic acid. Rabbit anti-fluorescein antibodies were prepared from rabbit anti-fluorescein serum¹¹ (the gift of Dr R. Cathou) in exactly the same way. Comparable results were obtained with both anti-fluorescein sera. The preparation of fluoresceinated sheep anti-rabbit IgG has been described previously³. The four types of cells studied were: normal rat mast cells, rat basophilic leukaemia cells, normal mouse mast cells and mouse mastocytoma cells (AB-CBF₁-MCT-1); their preparation has been described previously¹².

The principal experiments performed involved incubating the cells with a mixture (1:1-3:1) of F-IgE and R-IgE (total IgE concentration 10-15 $\mu\text{g ml}^{-1}$) with $\sim 1 \times 10^6$ cells ml⁻¹ for 60 min at 37 °C. The cells were washed once, taken up in one-tenth of the volume and reacted with $\sim 10 \mu\text{g}$ of anti-fluorescein (or anti-IgE) for 30-60 min at 37 °C and then examined with a Leitz Orthoplan microscope equipped with a Phloem incident light attachment, BG38 and KP490 exciting filters and a K530 suppression filter.

Cells pretreated with an excess of unconjugated IgE and then incubated as described always showed negligible green or red fluorescence. Cells reacted with the fluorescent IgE mixture and no anti-fluorescein or anti-IgE showed a diffuse distribution of both fluorescent chromophores (Table 1, experiment 1). The green fluorescence on cells reacted with the mixture of F-IgE and R-IgE and then with anti-fluorescein (Table 1, experiment 2) was distributed in multiple

Fig. 1 Three basic models of IgE receptor valency or integration which were explored by the experiments reported here. *a*, Receptors univalent and independent; *b*, receptors multivalent and independent; *c*, receptors univalent and connected. The experiments did not distinguish between *b* and *c* or a combination of *b* and *c*.

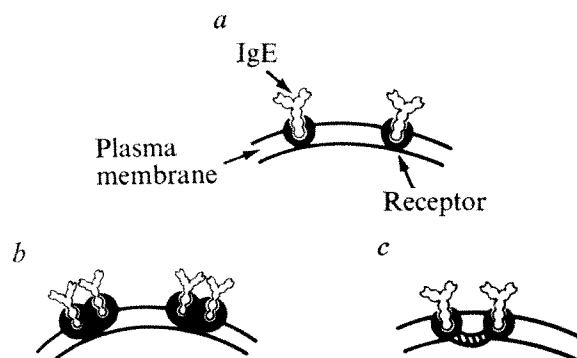


Table 1 Distribution of fluorescent IgE on rat and mouse cells

Type of experiment	Preincubation	Antiserum	Chromophore on IgE*	Topological distribution	
				Normal cells	Tumour cells
1	F-IgE + R-IgE	—	F R	diffuse diffuse	diffuse diffuse
2	F-IgE + R-IgE	anti-fluorescein	F R	small patches, vesicles diffuse	small patches, vesicles diffuse
3	F-IgE + R-IgE	anti-IgE	F R	comigration of F-IgE and R-IgE in small and large patches	comigration of F-IgE and R-IgE in caps and large patches
4	F-IgE	anti-fluorescein followed by R-IgE	F R	— —	small patches, vesicles† diffuse†
5	F-IgE	anti-IgE followed by R-IgE	F R	— —	caps and large patches, some vesicles† comigration with F-IgE plus diffuse†

*F, Fluorescein; R, rhodamine.

†Only mouse mastocytoma cells tested.

small irregular patches which appeared to be on the surface of the cells, as well as multiple smooth-bordered vesicle-like forms which appeared to be inside the cells. Some of the latter had a non-fluorescent centre, giving a "doughnut-like" distribution of fluorescence. In some cases the patches and vesicles were distributed asymmetrically on and in the cell but rarely were larger patches or caps observed. There was never any redistribution of the red fluorescence (R-IgE); cells reacted with anti-fluorescein or buffer were indistinguishable in this regard.

Cells reacted with NaN_3 (0.2 M) or at 4 °C during incubation with anti-fluorescein showed the F-IgE to be distributed in patches, but no vesicular forms were observed. When cells were first reacted with anti-fluorescein at 4 °C and the temperature was then raised to 37 °C, the vesicles appeared and became maximal within 15–20 min. As before, the red fluorescence never showed any redistribution.

Cells incubated with the F-IgE and R-IgE mixture and then with anti-IgE (Table 1, experiment 3) showed a different pattern; in all cases, the F-IgE and R-IgE comigrated. The basophilic leukaemia cells and mastocytoma cells showed extensive capping and large patch formation. The rat mast cells showed small or larger patches and only very rarely a fluorescent cap. The small mouse mast cells showed some cap formation but the larger cells showed only coarse aggregation. Vesicle formation was rarely seen with any of the cells. A similar picture was seen with cells reacted with IgE (unconjugated or conjugated), rabbit IgE and fluorescent sheep anti-rabbit IgE: the tumour cells showed large patches and caps whereas the mast cells showed principally patch formation; again little vesicle formation was observed.

These experiments demonstrated that receptors binding R-IgE did not comigrate with the receptors occupied by F-IgE when the cells were treated with anti-fluorescein, but did so when reacted with anti-IgE. We used mouse mastocytoma cells to test what would happen to empty receptors when receptors with IgE bound to them were redistributed. Cells were reacted with F-IgE such that when an aliquot was tested with ^{125}I -IgE only 40% of the sites were found to have been saturated. The partially saturated cells were reacted with antibody for 1 h at 37 °C, washed once and then reacted with excess R-IgE. Cells reacted with anti-fluorescein (Table 1, experiment 4) showed marked vesicle formation and some small patches for the green fluorescence but the red fluorescence was diffusely distributed. The green fluorescence on cells reacted with anti-IgE (Table 1, experiment 5) showed marked capping, and a small amount of vesicle formation (this was the only experiment in which vesicle formation was observed with anti-IgE). The R-IgE partially comigrated with the F-IgE

but was also diffusely distributed in areas between the green patches. We interpret this as the result of binding of R-IgE to non-patched empty receptors as well as to empty combining sites on anti-IgE in the patches formed by anti-IgE complexed to F-IgE. Control cells untreated with antibody showed a diffuse distribution of both F-IgE and R-IgE.

The results of our experiments are consistent with the observations on the surface distribution of IgE as assessed by transmission electron microscopy using ferritin-labelled antibodies^{3,5,13}. Those studies showed that in conditions in which the antibody itself would not be expected to perturb the system, the IgE was diffusely distributed. Although it was difficult to rule out oligovalency of the receptors, there was no evidence for even bivalency, and multivalency was clearly not observed. Those experiments could not of course elucidate whether there was any connection between receptors (Fig. 1c).

The present results seem, however, to be in conflict with those of Ishizaka and Ishizaka¹⁴, who carried out the following experiment: human basophils partially saturated with human IgE were reacted with anti-IgE in conditions which led to gross surface redistribution of the IgE. The cells were acidified in conditions where a substantial proportion of the cell-bound IgE-anti-IgE complexes seemed to be dissociated. The neutralised cells were then reincubated with radioiodinated IgE and the surface distribution of the radiolabelled IgE examined by autoradiography. Another portion of the cells was first saturated by added unlabelled IgE before the addition of the anti-IgE. Significantly, the two populations of cells showed a similar distribution of grains, over 50% of the cells showing either a capped or grossly asymmetric distribution of the label. These results, which suggested comigration of 'empty' receptors with receptors to which IgE was bound, were interpreted as "... that a group of receptor sites are associated with each other at the cell membrane or that receptor molecules on [the] basophil membrane are multivalent with respect to the combining sites for IgE"¹⁴. That is, models 1b and/or 1c were favoured: our data are only consistent with 1a (Fig. 1) and we are uncertain about the reason for this apparent discrepancy. Among the possibilities are (1) that the difference results from the use of cells from different species (human as opposed to rodent); (2) the use of different cells (basophils as opposed to mast cells); (3) that in spite of having several controls, the experimental results result from some artefact introduced by the nature of the protocol. The relative simplicity of our own experiments make us doubt that this has occurred in our studies, and so we conclude that, at least with regard to rodent mast cells

and related tumour cells, the receptors for IgE conform to the model depicted in Fig. 1a. We are not suggesting that the receptors are not interacting with other (sub-)membranous structures; indeed some results of studies on the diffusion of bound IgE in the plane of the membrane suggest that this may be true¹⁵. In the study by Schlessinger *et al.*¹⁵ it was similarly shown that the receptors for IgE are independently mobile and are monovalent.

Using non-ionic detergent it is possible to solubilise the cell-surface receptor for IgE in an active form¹⁶. Recent results indicate that such receptors are univalent with regard to their binding of IgE¹⁷. Together with the results reported here, these findings indicate that the receptor *in situ* is similar to the solubilised receptor, at least with regard to this important property.

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Lateral motion and valence of Fc receptors on rat peritoneal mast cells

MAST cells and basophils bind monomeric IgE antibodies reversibly but tightly by way of specific Fc receptors on their surface. Limited bridging of bound IgE molecules by multivalent antigen or anti-IgE antibody releases stored granules which liberate histamine and other mediators of the allergic response¹. The interaction of receptor-bound IgE with antigen (or with anti-IgE antibody) and the consequent cellular response (degranulation) provides an excellent opportunity for exploring the role of the lateral mobility of defined receptors in the transmission of 'signals' across the plasma membrane. Degranulation requires a limited bridging of complexes; excessive aggregation inhibits this response^{2–4}. We therefore measured the lateral mobility of the unperturbed IgE–receptor complexes on rat peritoneal mast cells and the effects on mobility of conditions that induce or inhibit degranulation. We redetermined⁵ the valence of the IgE receptor and the extent to which the IgE–receptor complexes react with each

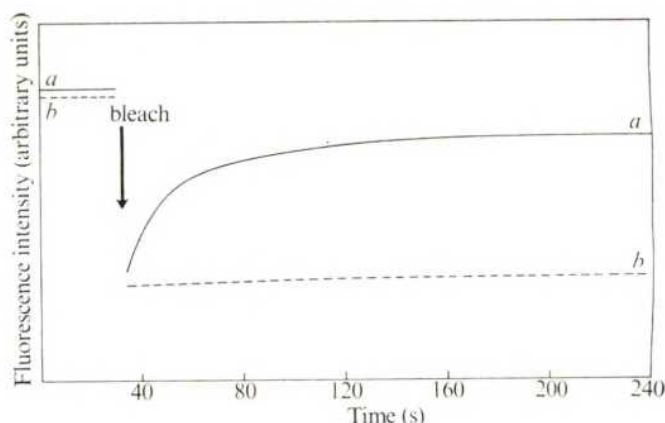
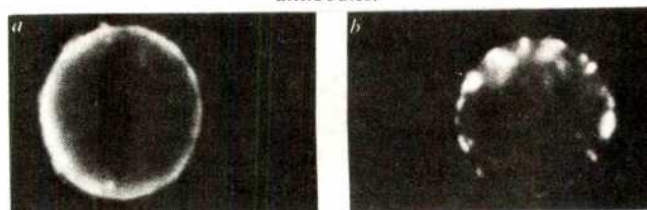


Fig. 1 Photobleaching recovery curves of mast cells labelled with *a*, R-IgE, $D = 1.8 \times 10^{-10} \text{ cm}^2 \text{ s}^{-1}$, f.r. = 75% (—); *b*, R-IgE and anti-IgE antibodies $D < 6 \times 10^{-12} \text{ cm}^2 \text{ s}^{-1}$, f.r. < 10% (---). In all experiments the small illuminated spot was exposed for 0.5 s to laser power of $\sim 1 \text{ mW}$. $D = (w^2/4\tau_{1/2}) \gamma$ where w is the e^{-2} radius of the focused Gaussian laser beam ($1 \mu\text{m}$); γ accounts for the amount of bleaching and the beam profile. In these experiments $\gamma \approx 1.3$ – 1.4 (ref. 8). Similar D and fluorescence recovery values were obtained for laser beam focused on the top or the bottom of the settled mast cells. Fixation with 3% glutaraldehyde for 1 h at 4 °C inhibited receptor motion.

other. Fluorescence photobleaching recovery (FPR) was used to measure the macroscopic lateral motion of fluorescent cell-surface components^{6–9}. We and others have used this or similar methods, to measure the mobility on cell surfaces of concanavalin A (con A) binding sites (which include various surface components) and nonspecifically labelled membrane proteins^{6,7,10–12}. The present work and a concurrent study of acetylcholine receptors on developing myotubes (Axelrod *et al.*, unpublished) are the first quantitative measurements of the lateral motion of specific cell-surface receptors.

The preparation of rhodamine- and fluorescein-labelled IgE (R-IgE and F-IgE) and of the antisera has been described elsewhere¹³, as has the procedure for obtaining the rat peritoneal mast cells¹⁴. The mast cells (at $10^6 \text{ cells ml}^{-1}$) were incubated with R-IgE or F-IgE or a 1:1 mixture of both, at a total IgE concentration of 20–30 $\mu\text{g ml}^{-1}$ for 45 min at 30 °C, then washed with Hanks' balanced salt solution (BSS), resuspended in 0.1–0.5 ml of BSS, and allowed to settle on glass microscope slides. In some experiments, 0.1 ml of mast cells preincubated with fluorescent IgE were treated with 10 μg of anti-fluorescein or anti-IgE antibody (sometimes in the presence of 0.01 M NaN_3 to prevent endocytosis) for 30 min at 37 °C, washed, and applied to a microscope slide. In FPR measurements a brief, intense, focused laser light pulse irreversibly bleaches the fluorescence in a small region ($\sim 3 \mu\text{m}^2$) of the membrane. The time course of recovery of fluorescence in the bleached region due to replenishment by fresh fluorophore from adjacent parts of the cell was recorded and the shape of the recovery curves showed that recovery is consistent with fluorophore diffusion on the cell surface⁸. Receptor

Fig. 2 Mast cells labelled with *a*, R-IgE; *b*, R-IgE and anti-IgE antibodies.



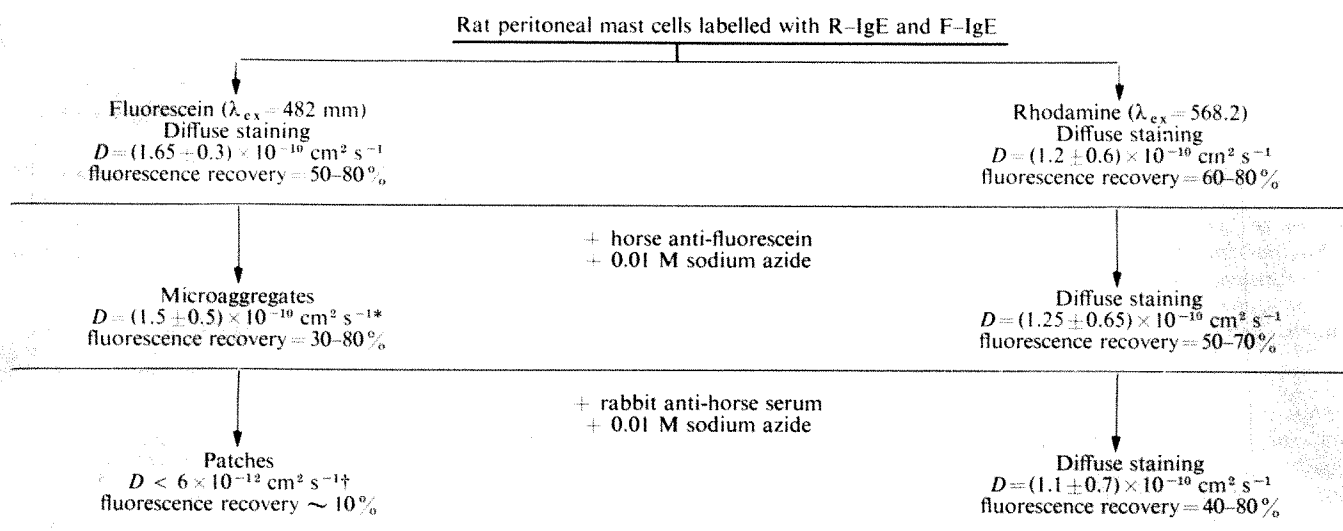


Fig. 3 Protocol for demonstration of IgE receptor valence. Mast cells were labelled with equal amounts of F-IgE and R-IgE. The values of D and f.r. and the fluorescence distribution were determined on individual cells independently for F-IgE and R-IgE by separate excitation of the two fluorophores using different excitation wavelengths. The left-hand column shows the protocol and results from detection of fluorescein; the right-hand column, from detection of rhodamine fluorescence on the same cell.

*Degranulation (G. Mendoza, unpublished).

†Inhibition of degranulation^{2,3}.

mobilities are, therefore, expressed as diffusion coefficients (D , $\text{cm}^2 \text{ s}^{-1}$) calculated from the recovery data. Incomplete fluorescence recovery is interpreted as indicating that a fraction of the fluorophores is immobile on the experimental time scale⁶⁻⁹. The fractional recovery (f.r.) therefore represents the fraction of mobile fluorophore⁶⁻⁹.

The mobility of dispersed IgE-receptor complexes is illustrated in Fig. 1, which shows a typical fluorescence recovery curve from a mast cell labelled with R-IgE. In this experiment, $D = 1.8 \times 10^{-10} \text{ cm}^2 \text{ s}^{-1}$ and the fraction of fluorescence recovery f.r. = 75%. The mean value and standard deviation for 17 cells was $D = (2.1 \pm 0.5) \times 10^{-10} \text{ cm}^2 \text{ s}^{-1}$ with f.r. in the range 50–80%. The division of the IgE-receptor complexes into mobile and immobile classes is interesting and although we cannot yet explain this heterogeneity, we have investigated factors which could influence it. We measured the diffusion coefficient of a fluorescent lipid probe (3, 3-diocadecylindocarbocyanine iodide⁷) in the plasma membrane of mast cells and found $D \approx 8 \times 10^{-9} \text{ cm}^2 \text{ s}^{-1}$, 40-fold greater than that of the IgE-receptor complexes. The phospholipid matrix is thus quite fluid, as has also been observed in rat myoblasts^{6,7}. The presence of immobile Fc receptors suggests that more than the fluidity of the phospholipid bilayer controls the receptor mobility. Sodium azide (10^{-2} M , 30 min) had no effect on fluorescence recovery, suggesting that the expenditure of metabolic energy was not involved. Colchicine (10^{-5} M , 45 min), which inhibits microtubule assembly, seemed to reduce f.r. to 20–50% but had no effect on D . In contrast, cytochalasin B ($25 \mu\text{g ml}^{-1}$, 45 min), which inhibits microfilament assembly, dramatically reduced the effective diffusion coefficient of Fc receptor to $D = (3.4 \pm 0.8) \times 10^{-11} \text{ cm}^2 \text{ s}^{-1}$, and reduced the f.r. to 20–50%. These three substances do not affect the diffusion of a lipid probe in either myoblast plasma membranes or planar lipid bilayers, demonstrating that the viscosity of the lipid matrix was not altered by the drug treatments⁷. Therefore we conclude that cytoskeletal interactions, especially with microfilaments, can influence the receptor mobility^{6,7}.

Treatment of mast cells prelabelled with F-IgE with a 10- to 50-fold excess (over receptor) of anti-fluorescein antibodies causes degranulation (G. Mendoza, unpublished). FPR measurements of the diffusion of F-IgE in these conditions yielded $D = (1.5 \pm 0.5) \times 10^{-10} \text{ cm}^2 \text{ s}^{-1}$ with f.r. from 40% to

80%. Fluorescent microaggregates, each $< 1 \mu\text{m}$ incorporating a substantial part of the total fluorescence, were visible on the cell surface. Similar results were obtained two hours after labelling. Aggregation of IgE to a level adequate for degranulation did not therefore, cause a detectable change in mobility. We cannot, however, exclude effective immobilisation of a small fraction of F-IgE.

In contrast, incubation of cells with anti-IgE antibodies (rather than anti-fluorescein) following labelling with F-IgE or R-IgE caused extensive redistribution of the complexes and large patch ($> 1 \mu\text{m}$) formation (see Fig. 2). FPR measurements carried out soon (15 min) after labelling indicated that the crosslinked receptors were essentially immobile; $D < 6 \times 10^{-12} \text{ cm}^2 \text{ s}^{-1}$, f.r. $< 10\%$. Diffusion of receptors is thus unaffected by the limited crosslinking by anti-fluorescein that is adequate for degranulation. Diffusion is, however, strongly reduced in conditions in which degranulation is inhibited by excessive aggregation with anti-IgE^{2,3}.

In the accompanying paper, Mendoza and Metzger¹³ show that anti-fluorescein antibodies induced redistribution only of F-IgE cells prelabelled with both R-IgE and F-IgE and so they concluded that the Fc receptor is monovalent for IgE. We have devised another sensitive experiment (Fig. 3) to test this conclusion, in which mast cells were labelled with equal amounts of F-IgE and R-IgE. The mobility of the fluorophores could be measured separately using krypton laser lines at 482 nm and 568.2 nm respectively. As expected, horse anti-fluorescein antibodies affected the mobility of neither F-IgE nor R-IgE. Addition of rabbit anti-horse antibodies, which bind only to the anti-fluorescein antibodies, caused patching and abolished the mobility of the F-IgE without diminishing the mobility of the R-IgE (see Fig. 3). This reinforces the conclusion of Mendoza and Metzger¹³ that the Fc receptor is monovalent and rules out the possibility that individual receptor molecules interact or are bound together to a significant degree⁶.

The major conclusions of the present study are: (1) Most IgE-Fc receptor complexes are mobile with $D = (2.1 \pm 0.5) \times 10^{-10} \text{ cm}^2 \text{ s}^{-1}$, but 20–50% are immobile on the experimental time scale. (2) Limited crosslinking by anti-fluorescein that is adequate for degranulation did not affect the apparent mobility of the receptors. Extensive aggregation inhibitory of degranulation, however, abolished the mobility. (3) The Fc receptor is monovalent for IgE. (4) Cytochalasin B reduces the

lateral mobility of the complexes; colchicine has little detectable effect.

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Induction of an IgM anti-(bovine)-IgG response in mice by bacterial lipopolysaccharide

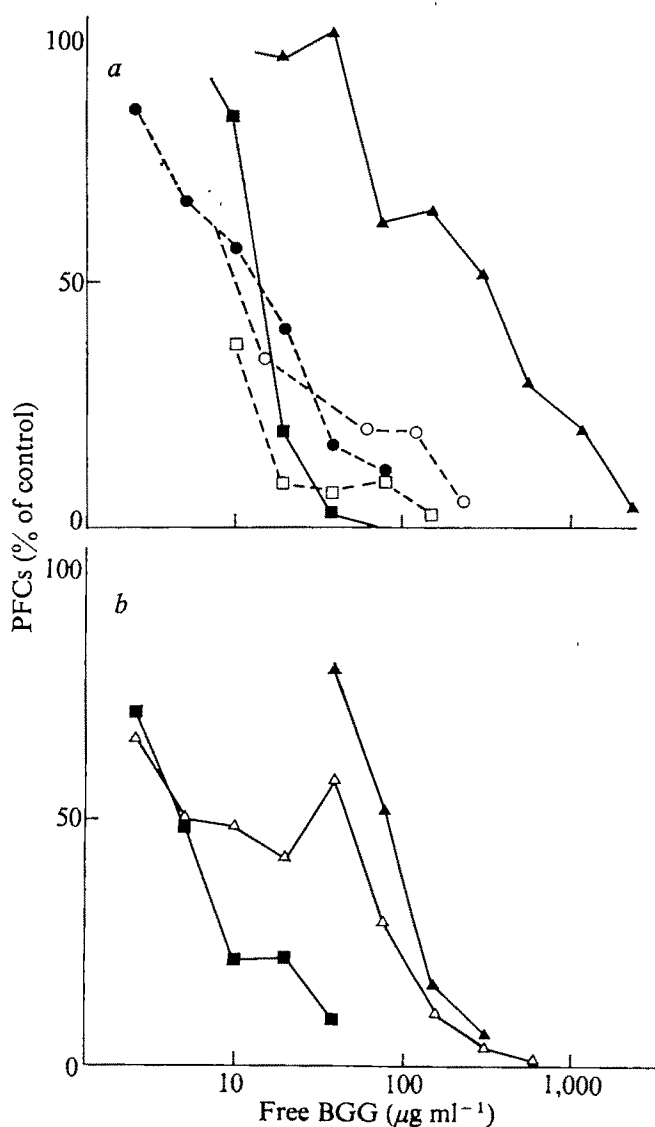
BACTERIAL endotoxin stimulates the appearance in mice of a large number of plaque forming cells (PFC) secreting an IgM globulin with specificity for bovine IgG (bovine gamma globulin; BGG). Reversed plaquing (antibody on the target erythrocyte) has enabled us to enumerate the total number of IgM secreting cells and show that the anti-BGG PFC comprise at least 10% of the total. Plaque inhibition with free antigen shows that anti-BGG PFC stimulated by LPS, as distinct from those specifically stimulated, secrete a product with low avidity. The evidence reported both here and in a previous study¹, leads us to suggest that substances such as LPS stimulate the production of an M-anti-G response. The rheumatoid-factor-like molecule which is produced may possibly serve to amplify complement fixation by high avidity/specificity IgG antibodies.

The injection into mice of certain substances apparently unrelated to sheep red blood cells (SRBC), can increase the level of 'background' antibody to SRBC, or the number of plaque forming cells producing antibody to SRBC²⁻⁴. Of such substances⁵ investigated, *Bordetella pertussis* (pertussis) organisms and water-in-oil emulsions containing heat killed mycobacteria (Freund's) can be readily classified as immunological adjuvants, and rabbit anti-(mouse)-lymphocyte globulin (ALG) as a mitogen lipopolysaccharide endotoxins from Gram-negative bacteria (LPS) combine the properties of both⁴⁻⁶. LPS stimulates not only an increase in cell growth and division *in vitro* but also the development of cells secreting antibody to SRBC and to the haptens NIP and NNP⁷. Since the mitogenicity of LPS is

confined to B-lymphocytes and since it also stimulates the production of antibodies with a wide range of specificities, including those specific for the antigenic moiety of LPS itself⁸⁻¹⁰, Möller and his colleagues coined the term PCBA—polyclonal B-cell activator—to describe substances with the properties just ascribed to LPS¹¹.

In the course of experiments designed to investigate the induction of tolerance to BGG in CBA/Ca mice, some animals received either an intraperitoneal injection of alum adsorbed BGG plus pertussis or an intravenous injection of

Fig. 1 Inhibition of anti-BGG plaques by free BGG added to the medium of the plaque assay. Each assay consists of 0.2 ml 1% Agarose-Eagle's basal medium plus target erythrocytes, PFC, and developing serum, spread into an area of about 25 × 50 mm on a standard microscope slide. *a*, Solid lines refer to plaques developed by a μ -specific developing serum on slides carrying spleen cells from CBA/Ca mice injected intraperitoneally 4 d before with either 20 μ g LPS (\blacktriangle ; 100% = 31×10^4 PFC/spleen) or intravenously with 1 mg HBGG (\blacksquare ; 100% = 2.3×10^4 PFC/spleen). The dashed lines refer to three separate experiments in which plaques were developed with a γ_1 -specific developing serum¹² using spleen cells taken 4 d after injection of HBGG (mean 100% = 1×10^4 PFCs per spleen). The solid symbols refer to data obtained on the same day. Each pool of spleen cells was prepared from five 8-10-month-old male mice. *b*, As for (*a*) except that all the data refer to anti- μ developed plaques. Mice were injected with either LPS (\blacktriangle ; 100% = 7.5×10^4 PFC per spleen), HBGG (\blacksquare ; 100% = 4.7×10^4 PFCs per spleen) or HBGG plus LPS (\triangle ; 100% = 19.3×10^4 PFC per spleen).



heat aggregated BGG (HBGG). The immune response of these mice was measured using a haemolytic-plaque assay, using as target cells, SRBC and SRBC coated with BGG by the chromic chloride method^{12,13}. When a rabbit anti-(mouse)- μ serum was being used in an attempt to inhibit direct plaques, it was found that this serum would enhance by about fourfold the number of plaques against BGG coated SRBC: the concentration of anti- μ serum used was one which had already been shown to be highly inhibitory to direct plaque formation by anti-SRBC PFC from the spleens of actively immunised mice. The developed plaques, which are very small, are an example of indirect IgM PFCs^{14,15}.

Subsequently, it has been found that such indirect IgM anti-BGG responses can be stimulated in CBA/Ca mice by the injection of pertussis alone, and even more so by LPS (*Salmonella typhosa* 0901 B). The maximum response is found 4 d after an intraperitoneal injection of 20 μ g of LPS.

The (indirect) anti-BGG PFC stimulated by LPS are judged to be IgM-producers on the following criteria. (1) Their numbers are enhanced by a rabbit anti-(mouse)- μ serum, raised, absorbed and tested for specificity as described previously¹⁶. Another such serum, kindly provided by Dr R. M. E. Parkhouse, gave an identical enhancement, which was abolished by passage through a μ -bearing Sepharose 4B column. (2) A goat anti-(mouse)- μ serum has been prepared which inhibits totally both direct anti-SRBC and LPS stimulated anti-BGG plaques. The difference in behaviour between the rabbit and goat anti- μ sera may be explicable on the basis of their differing abilities to fix the guinea pig complement used in the assay¹⁷. (3) The indirect anti-BGG plaques are ablated by reduction and alkylation procedures designed to destroy IgM but not IgG¹⁴.

The anti-BGG response, nonspecifically induced by LPS, is restricted in duration and in Ig class, although the number of PFC present in the spleen at the peak of the response, 4 d after the intraperitoneal injection of LPS, is quite high ($>10^5$ PFC per spleen). In contrast, the anti-BGG response, specifically induced by intravenous injection of HBGG, produces far fewer PFC per spleen ($<10^3$), and in addition induced both an early IgG (γG_1) peak 5 d after the injection, and a late IgG (γG_1 , γG_{2a} , γG_{2b}) peak 10 d after the injection of HBGG. The avidity for BGG of the antibody produced by PFC in the two types of response, has been compared (Fig. 1). A relative avidity can be deduced from the amount of free BGG required to reduce plaque numbers by an arbitrary proportion¹⁸, say to 32% of the

numbers detected in its absence. All PFC, irrespective of class, which were specifically induced by HBGG are inhibited by far lower concentrations of BGG than are the indirect PFC induced nonspecifically by LPS: it is concluded that the antibody induced by LPS has a very low affinity (avidity) for BGG. It is interesting to note that immunisation by a mixture of LPS and HBGG leads to an exceedingly heterogeneous population of PFC as judged by the shallow slope of the inhibition curve (Fig. 1b). This suggests that although they may overlap extensively, LPS and HBGG tend to stimulate different bands in the spectrum of precursor cells capable of becoming anti-BGG PFC. The nature of the inhibition curves and the numbers involved provide evidence that LPS stimulates a very much larger number of precursor cells than does HBGG. The possibility that LPS and HBGG stimulate some of the same precursors cannot be excluded on the evidence.

It is debatable whether LPS triggers B lymphocytes through being bound to Ig receptors for antigen. With the proviso that the polysaccharide (antigenic) moiety probably behaves like any other antigen, the lipid A (adjuvant) moiety may bind either to Ig receptors for antigen at positions other than the antibody-like site or, alternatively, to other (non-Ig) sites on the cell membrane. The triggering reflex may be the result of a cumulative effect exceeding some unspecified threshold, which results from the binding and perhaps crosslinking of a variety of membrane components: antigen, LPS and certain factors of lymphocyte or macrophage origin may well help to exceed this threshold. An hypothesis which suggests that antigen and adjuvant give qualitatively different triggering stimuli to antigen sensitive cells (ASC), thereby deciding between tolerance and immunity, has been discussed elsewhere¹⁹.

In passing, it may be worth noting the following points additional to the apparent differences in relative avidity of the IgM responses to BGG. (1) HBGG but not LPS stimulates an IgG response. (2) Secondary IgG (IgM?) responses stimulated by BGG after priming with HBGG can be demonstrated, but it has not been possible to demonstrate that LPS stimulates a secondary IgM response in mice primed either by HBGG or LPS. (3) In "tolerance" induction large doses of BGG (>1 mg) are required to reduce the level of IgM response but much smaller doses (<10 μ g) to reduce the level of IgG response¹³. (4) The magnitude of the peak anti-BGG response stimulated by LPS increases with increasing age of the mice up to about 8 months and thereafter remains more or less constant. There are no

Table 1 The proportion of cells secreting IgM with antibody-like properties 4 d after an injection of LPS

Experiment number	Arithmetic mean number of cells or (IgM) PFC per spleen ($\times 10^4$)					
	Cells*	IgM secretors (indirect)	Anti-BGG (indirect)	Anti-sheep IgG (indirect)	Anti-TNP† (direct)	Anti-SRBC (direct)
Normal mice (not injected)						
1	11,600	13	0	0	1.7	0
LPS injected mice						
1	16,800	820	75	11	47	1.8
2	17,500	314	62	—‡	13	0.1
3	22,600	522	32	—	—	2.9
4	14,100	224	35	1.6	33	0.4
5	13,400	1054	34	—	—	1.1
6	10,700	—	24	—	—	—
7	10,800	874	—	—	—	—
8	13,800	178	13	—	—	0.4
Mean	14,963	569	39.3	—	31	1.1
(\pm s.e.)	(1,389)	(132)	(8.2)	—	(9.9)	(0.5)

*Cell counts were made with a model B Coulter counter windowed to exclude erythrocytes or smaller cells and cells the size of large blast cells or larger. As judged by Trypan blue exclusion cell viabilities are $>77\%$.

†SRBC coated with TNP at high epitope density by the method of Rittenberg and Pratt²¹.

‡A dash means that this target cell was not used in this experiment.

On the basis of these data, $4.0 \pm 0.43\%$ of spleen lymphocytes are secreting detectable amounts of IgM: of the IgM secretors $10.2 \pm 1.0\%$ are secreting anti-BGG antibodies, $8.2 \pm 1.9\%$ are secreting antibodies binding to TNP and only $0.22 \pm 0.07\%$ are secreting anti-SRBC antibody.

measurable changes in avidity to BGG of the IgM response induced by LPS over the age range of 2–20 months.

An estimate has been made of the total number of IgM secreting cells in the spleens of mice, both before and after the injection of LPS. The estimate was made using the technique of "reversed-plaquing"²² in which the target erythrocytes used in the plaque assay were coated with IgG from a goat anti-(mouse)- μ serum. An essential step was to develop the plaques by use of a rabbit anti-(mouse)- μ serum at the concentration used to enhance the level of anti-BGG plaques. Table 1 summarises the results of several experiments in which estimates have been made of the number of IgM secreting cells and the proportion of these cells which were secreting antibody reactive with BGG, sheep IgG, SRBCs and the hapten 2,4,6-trinitrophenyl (TNP). It is striking that, on the basis of these measurements, the anti-BGG response comprises about 10% and the anti-TNP about 8% of the total number of IgM secreting cells.

We think it is possible that the IgM response to BGG induced by an injection of LPS represents an unconventional immune response in which rheumatoid-like factors^{22,23} are secreted in response to stimulation by toxic products released by bacteria. In favour of this hypothesis are the facts that the response is IgM, low avidity and directed against an IgG molecule. The implication would be that to the CBA mouse, bovine IgG and bound or denatured murine IgG have similar antigenic determinants. So far however our attempts to demonstrate this directly using the plaque assay have been inconclusive. Nevertheless it has been possible to demonstrate that LPS stimulates the production of a humoral IgM (19S) which binds with relatively low avidity to mouse (γ G_{2a}) antibody bound to antigen (SRBC)¹. If our reasoning is correct, the high proportion of IgM secreting cells whose products have anti-IgG activity, could be regarded as a fast amplification mechanism which might be an adjunct to the complement cascade. The selective pressures influencing the evolution of this mechanism would therefore be quite different from those moulding high avidity responses to foreign determinants with a wide range of specificities.

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Immunological response restricts number of cells in sensory ganglia infected with herpes simplex virus

INFECTION of mice with herpes simplex virus (HSV) by various epithelial routes has been shown to result in a latent infection of the corresponding sensory or autonomic ganglia^{1–3}. During the acute phase of the infection (less than 14 d), virus could be recovered from ganglia both by homogenisation and explantation, while during the latent phase of the infection (21 d to more than 1 yr), virus could be recovered only by explantation and not by homogenisation. Although these methods made it possible to tell whether ganglia were infected, they gave no information about the percentage of ganglionic cells containing virus. We have therefore investigated the number of cells in sensory ganglia infected with HSV, and evaluated the influence of immunity on the severity of the infection.

HSV, type 1 (strain CHR-3), containing 2×10^8 plaque-forming units (PFU) per ml was prepared and assayed in primary rabbit kidney (PRK) cells⁴. Swiss athymic nude mice (Nu/Nu), normal littermates (Nu/+ or +/+) and BALB/c mice were infected with HSV by administering undiluted stock virus to the abraded right rear footpad². BALB/c mice were immunised with HSV by administering increasing doses of infectious virus (10^3 – 10^6 PFU) intraperitoneally at 10–14-d intervals, over 2 months. Previous experiments showed that the dorsal root ganglia (DRG) of mice did not become infected as a result of intraperitoneal immunisation⁵. Hyperimmune serum to HSV was prepared in rabbits by repeated intradermal injection (0.5 ml) of partially purified virus⁶. Neutralisation titres are expressed as the reciprocal of the highest dilution of serum producing a 50% reduction in viral plaques.

To determine the number of ganglionic cells infected with HSV, DRG were removed, washed in Dulbecco's phosphate-buffered saline (PBS) and incubated at 36 °C for 15 min in 2.0% collagenase (approximately 10 ganglia per ml). Tissue was aspirated gently four or five times with a Pasteur pipette, washed three times in trypsin-EDTA (0.25% trypsin in 4×10^{-4} M EDTA-saline), and then incubated at 36 °C for 15 min in the trypsin-EDTA solution. Foetal bovine serum was added to a final concentration of 10%. To eliminate extracellular virus, the ganglionic cells were

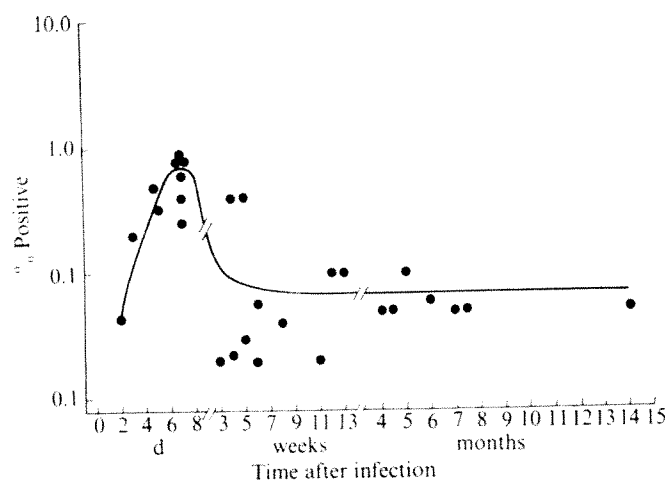


Fig. 1 Percentage of ganglionic cells infected with HSV at different times after infection. BALB/c mice were inoculated with HSV by the footpad route and DRG were removed and dissociated into single cells. Serial twofold dilutions of the cell suspension were placed on PRK cell monolayers and the percentage of infected ganglionic cells was determined.

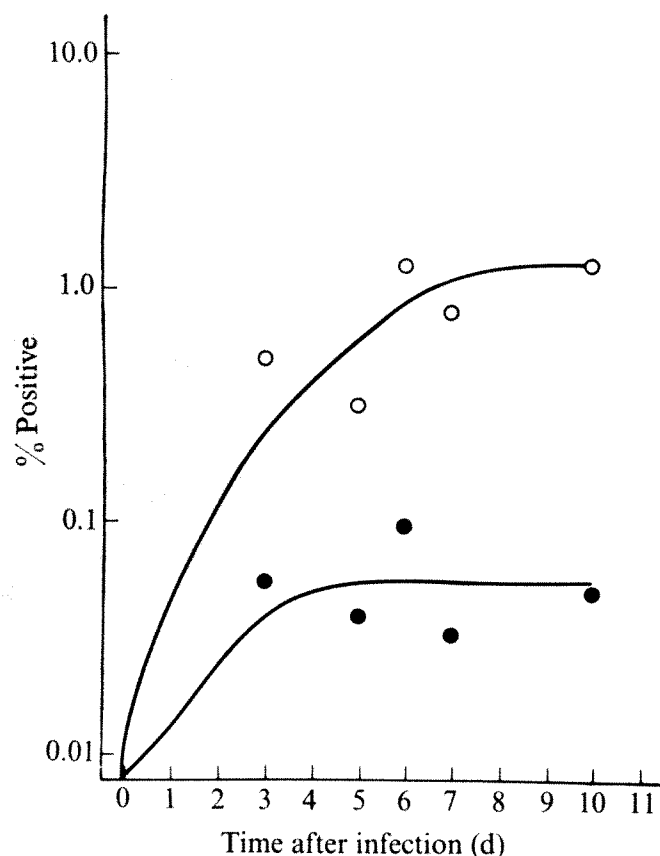


Fig. 2 Effect of immunisation on number of cells in DRG infected with HSV. Immunised (●) and unimmunised (○) BALB/c mice were inoculated by the footpad route with HSV and at different times thereafter DRG were removed and dissociated and the percentage of infected cells was determined.

resuspended and incubated for 30 min in rabbit anti-HSV serum (50% neutralisation titre, 400). The cell suspension, containing primarily single cells, was washed five times to remove excess neutralising antibody, resuspended in PBS, and the viable cells were counted by Trypan blue exclusion. Based on staining for Nissl bodies⁷, we estimated that the final cell suspension contained 5–10% neurones. Supernatant fluid from the fifth cell wash was assayed to ensure that infectious virus was not present in the final cell suspension. Serial twofold dilutions of the cell suspension were placed on triplicate or quadruplicate monolayers of PRK cells. Cultures were examined daily for 10 d for the appearance of HSV cytopathology. Percentage of ganglionic cells infected was calculated (according to the method of Reed and Muench) by determining the number of dispersed ganglionic cells required to produce lysis of the PRK monolayers in 50% of the cultures (that is, based on the assumption that only one infected ganglionic cell was needed to initiate lysis).

DRG were removed from animals at different times after footpad inoculation with HSV, and the number of infected cells in ganglia was determined. Figure 1 shows that between 6 and 8 d after inoculation, almost 1.0% of the cells in the ganglia were infected. During the next few weeks, the percentage of infected cells decreased to 0.1% or less, and remained at that level for almost 15 months.

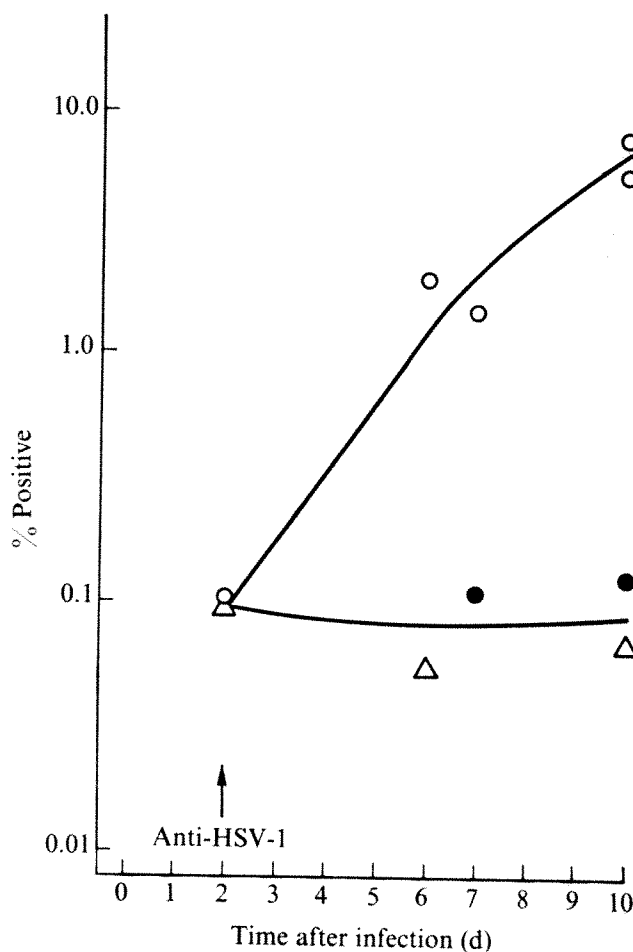
Earlier experiments showed that immunisation with HSV did not prevent mice from developing a latent infection when challenged by the footpad route³. To see whether immunisation affected the total number of ganglionic cells that became infected, immunised and unimmunised mice were challenged with HSV by the footpad route, and at different times thereafter the virus-infected cells in ganglia were counted. The data in Fig. 2 show that immunisation

restricted infection of ganglionic cells; approximately one-tenth the number of ganglionic cells was infected in immunised as in unimmunised animals. Moreover, the number of infected cells in ganglia of immunised animals during the acute phase of infection corresponded closely to the number of infected cells in ganglia of unimmunised animals during the chronic phase of the infection (Fig. 1).

Further evidence that the immune status of the host influences the number of cells infected in ganglia comes from studies with nude mice. Figure 3 shows that the number of infected cells in ganglia of nude mice (Nu/Nu) continued to rise during the first 10 d of the infection and reached close to 7.0%. In contrast, only 0.1% of ganglionic cells was infected in nude mice given antibody to HSV 2 d after HSV inoculation. Similarly, only 0.1% of the ganglionic cells was infected in phenotypically normal littermates of nude mice (Nu/+ or +/+) not given antibody to HSV. Thus, the nude mouse, which cannot mount an effective immune response to HSV⁸, has almost 70 times more HSV-infected cells in the sensory ganglia than the antibody-reconstituted nude mouse (Nu/Nu) or the phenotypically normal mouse (Nu/+ or +/+).

The studies described here show that by dissociating sensory ganglia, it is possible to determine the number of cells

Fig. 3 Percentage of ganglionic cells infected with HSV in immunologically deficient animals. Nude mice (Nu/Nu) and phenotypically normal littermates (Nu/+ or +/+) were inoculated by the footpad route with HSV. Two days later the nude animals were given intraperitoneally either 0.1 ml of normal mouse serum or 0.1 ml of mouse anti-HSV serum (50% neutralisation titre, 1,048). At different times thereafter DRG were removed and dissociated and the percentage of infected cells was determined. ○, Nude mice given normal mouse serum; ●, nude mice given mouse anti-HSV serum; △, phenotypically normal littermates of nude mice.



infected by HSV. Because of the potentially low plating efficiency of infected cells, as well as the possible loss of cells due to physical manipulation, the actual number of virus-infected cells in ganglia may be considerably greater than the 0.1–1.0% obtained in our experiments. Moreover, although our data are expressed as percentage of cells in ganglia infected with HSV, it was estimated that less than 10% of the cells plated were neurones.

Even though the assay used here may greatly underestimate the true number of cells infected, it does provide a useful method for comparing the relative number of cells infected in different experimental conditions. In this connection, we found that the number of infected ganglionic cells fell off sharply after the acute phase of the infection and remained relatively constant for many months thereafter. Whether this is due to two populations of cells in the ganglion susceptible to HSV—one capable of being lysed by the virus, and the other capable of maintaining a latent infection—or whether the immune response of the host destroys some of the virus-infected cells is not known. Our studies with immunised and nude mice, however, do show that the immunological status of the host is one of the factors that controls the number of cells in ganglia that becomes infected. Precisely how the immune response of the host restricts infection of ganglionic cells is not clear. It is known that HSV replicates in the skin and travels up the nerve to the cell body in the ganglion^{9–11}. Thus the number of nerve endings exposed to the virus may determine the number of cells ultimately infected. If the immune response of the host inhibits viral replication in the skin, the number of cells becoming infected would be reduced correspondingly. Alternatively, the virus may spread from one cell to another within the ganglion and the humoral and/or cellular immune response may act at this level¹². The demonstration in nude mice that passive administration of antibody is protective, suggests that humoral immunity plays a part in limiting the initial spread of the virus. Whether humoral immunity also is responsible for maintaining the latent state of the infection¹³ cannot be resolved from our experiments.

The demonstration that, in different experimental conditions, the number of infected ganglionic cells varies considerably, suggests that this might be an important factor in determining the subsequent clinical course of the disease. All other things being equal, the severity and frequency of recurrent HSV attacks might very well be related to the number of latently infected cells.

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A human chromosomal determinant for susceptibility to herpes simplex virus

THE susceptibility of mammalian cells to virus infection has been studied in many virus–host systems. In certain instances^{1–6}, the comparison of cells which do not permit productive infection with those that do has made it possible to identify the host factors which allow entry of the virus and support its subsequent growth. In the case of poliovirus the use of somatic cell hybrids between permissive and non-permissive cells has led to the assignment to human chromosome 19 of a gene coding for virus susceptibility⁷. Although cells from a wide variety of organisms, including many rodents and man, are susceptible to lytic infection by herpes simplex virus type 1 (HSV-1) resistance to HSV-1 infection has been reported in certain Chinese hamster cell lines^{8–10}, in dog kidney cells¹¹, and in a line of rat cells¹². In these cell lines HSV-1 is either non-infectious or exhibits virus titres several orders of magnitude lower than in Syrian hamster or rabbit cells. The cellular factors involved in this resistance to infection and in the genetic control of susceptibility to HSV-1 have not yet been identified.

We report here experiments in which Chinese hamster × Syrian hamster and Chinese hamster × human somatic cell hybrids were used as hosts for HSV-1 infection. The results indicate that susceptibility to HSV-1 infection behaves as a partially dominant genetic trait in these cells, and that its behaviour in Chinese hamster × human hybrids which are segregating human chromosomes is consistent with its being determined by the presence of human chromosome 3.

The results in Table 1 confirm the earlier finding⁹ that HSV-1 has low infectivity in Chinese hamster Don cells¹³ and show that genetically marked derivatives of Don are also less susceptible to HSV-1. As certain of the cell lines were unable to form complete monolayers, we used the 17 *syn* strain¹⁴ of HSV-1 in most of our experiments. This strain causes polykaryocytosis which results in the formation of easily recognised syncytial plaques. Where tested, essentially similar results were obtained with the *syn*⁺ parent strain. As can be seen, virus titres were at least 10⁴-fold lower on Don and its derivatives than on Syrian hamster BHK-C13 cells¹⁵. Reproducible virus titres on Don cells could only be obtained at multiplicities above 0.1 PFU per cell (as measured on BHK-C13 cells). The burst size in these conditions as measured by infectious centres and yield assays (approximately 150 PFU per infected Don cell) was comparable to that on BHK-C13 cells. The progeny virus

Table 1 Susceptibility of Syrian hamster and Chinese hamster cell lines to HSV-1 strains

Cell line	Virus titre (log ₁₀ PFU* ml ⁻¹)	
	HSV 17 <i>syn</i>	HSV 17 <i>syn</i> ⁺
BHK-C13	7.3	9.3
C13 TK ⁻	7.3	9.6
Don	2.3	5.2
Don TG 5.10 [‡]	2.5	5.6
Don TK ⁻ (a23)§	2.5	ND

Monolayers of each cell line in 50-mm plastic Petri dishes were infected with HSV 17 *syn* (low titre stock passaged in Lesch-Nyhan GM29 cells) or HSV 17 *syn*⁺ (high titre stock passaged in BHK-C13 cells). After 40 min incubation at 37 °C in Glasgow-modified Eagle's medium supplemented with 10% foetal calf serum, the inoculum was neutralised by the addition of an equal volume of medium containing 10% human serum. The neutralised inoculum was removed after a further 20 min incubation at 37 °C and replaced by medium containing 10% human serum. Incubation was continued for a further 48 h at 37 °C. Dishes were stained with Leishman stain and virus plaques counted. ND, not determined.

*Plaque-forming units.

[†]Isolated by Littlefield and Basilico¹⁶.

[‡]Isolated in this laboratory from Don cells by selection in 5 µg per ml 6-thioguanine.

[§]Isolated by Dr A. Westerveld¹⁹, and supplied by him.

Table 2 Susceptibility of Syrian hamster \times Chinese hamster somatic cell hybrids to HSV-1

Clone	Virus titre (log ₁₀ PFU ml ⁻¹)	Modal chromosome no.	Don contribution to hybrid cells
BHK-C13 TK ⁻	7.2	41 (36-45)*	—
Don TG 5.10	2.7	44 (42-46)†	—
A1	5.0	60 (45-65)	1s‡
A5	5.3	65 (65-72)	1s
A6	4.6	62 (55-71)	1s
B3	5.0	100 (88-104)	1s
B5	5.3	61 (55-65)	1s
C1	6.0	86 (75-95)	1s

BHK-C13 TK⁻ and Don TG 5.10 (HGPRT⁻) cells were fused in monolayer¹⁷ using ultraviolet-inactivated Sendai virus (Searle Diagnostic Ltd), and complementing hybrid colonies isolated in modified²⁰ HAT medium¹⁸. Independent colonies were picked after 14 d, grown in plastic flasks (Nunc) to a total population size of 5×10^7 cells, and aliquots taken for karyotype and isozyme analyses or for storage in liquid N₂. Virus susceptibility tests were performed using HSV 17 *syn* as described in Table 1, on thawed cells which had been grown for one further passage. Chromosome numbers and the complement of Don chromosomes were determined by examination of Giemsa-banded²⁰ metaphase spreads.

*Range of values (90% limits).

†Don TG 5.10 is tetraploid.

‡1s indicates the presence of not more than 44 Chinese hamster chromosomes in the majority of cells.

produced did not, however, show an increased titre on Don cells.

The molecular basis for the low susceptibility of Don cells to HSV-1 infection is at present being investigated. Results not shown here indicate that, although adsorption and penetration of virus occur normally, there is little or no virus eclipse and viral thymidine kinase is not induced. We cannot at present, however, exclude the possibility that some early viral proteins are synthesised in Don cells infected at low multiplicity.

In order to determine whether susceptibility to HSV-1 is a genetically dominant trait, somatic cell hybrids were made between non-permissive and permissive cells. Cells of a Don HGPRT⁻ variant (Don TG 5.10) and a BHK-C13 TK⁻ variant were fused and complementing hybrids between them isolated under conditions designed to provide a series of hybrid clones of independent origin. These clones were tested at low multiplicities for their susceptibility to HSV-1. Table 2 shows that the Don \times BHK hybrids were susceptible to HSV-1 and gave virus titres 10^2 – 10^3 -fold less than the BHK parent. This result indicates that susceptibility

Table 3 Susceptibility of Chinese hamster \times human somatic cell hybrids to HSV-1

Clone	Virus titre (log ₁₀ PFU ml ⁻¹)	Susceptibility
Parental lines		
GM29*	6.5	+
Don TK ⁻ (a23)	2.3	—
Hybrids		
GMA A1.5	2.8	—
GMA A2.5	4.2	+
GMA C1.3	2.5	—
PF 1B6	<2.0	—
PF 2A5	4.3	—
PF 2B1	3.3	+
PF 2B2	3.3	+
PF 2C2	<2.0	—
PF 4A1	<2.0	—
PF 4A2	4.0	+
PF 4A6	<2.0	—
PF 4B1	<2.0	—
PF 4B2	4.6	+

Lesch-Nyhan GM29 (HGPRT⁻) and Don TK⁻ (a23) cells were fused in monolayer (PF series) or suspension (GMA series) using ultraviolet-inactivated Sendai virus¹⁷. Complementing hybrids were isolated and analysed as in Table 2.

*Purchased from the Mammalian Genetic Mutant Cell Repository, Camden, New Jersey.

to HSV-1 behaves as a partially dominant trait. Although preferential loss of one parental set of chromosomes is not expected from these hybrids, it is clear that in some cases the average chromosome number in the hybrids is less than the sum of those of the two parents. Although we are unable to state conclusively that this was not due to preferential loss of chromosomes of one parent, there is no correlation between the extent of chromosome loss and susceptibility to HSV-1.

Hybrids were also constructed between a Don TK⁻ variant (a23) and human HGPRT⁻ skin fibroblasts from a Lesch-Nyhan patient (GM29). In this case the preferential and random loss of human chromosomes from such hybrid clones is well established¹⁹. A series of independently isolated Chinese hamster \times human hybrid clones were assayed for HSV-1 susceptibility as before (Table 3). As can be seen, partial dominance of susceptibility was demonstrated by six of the hybrid clones, while the remaining seven clones were non-susceptible. The susceptible clones gave titres approximately 10^2 – 10^3 -fold less than that of the human parent, which is in agreement with the 10^2 – 10^3 -fold difference between Don \times BHK hybrids and their BHK parent, whilst virus titres on the non-susceptible clones were similar to those obtained on Don. This result implies the existence of a partially dominant human chromosomal determinant for HSV-1 susceptibility which segregates out of the hybrids as human chromosomes are lost.

In order to determine the complement of human chromosomes

Table 4 Correlation between the presence of human chromosome 3 and HSV-1 susceptibility in Chinese hamster \times human somatic cell hybrids

Chromosome 3	HSV-1 susceptibility	
	+	—
+	6	0
—	0	7

Giemsa-banded metaphase spreads of Chinese hamster \times human hybrid clones were prepared as described previously²⁰. A minimum of 25 metaphase cells of each clone were scored for the presence of human chromosome 3; positive clones were those in which chromosome 3 was present in more than 50% of cells. The data on HSV-1 susceptibility were taken from Table 3. The table shows the number of clones found in each category.

in the hybrid clones, isozyme^{21,22} and karyotype analyses were performed. Using the technique of Cellophane electrophoresis the hybrids were analysed for the following isozyme markers: phosphoglucose mutase-1, isocitrate dehydrogenase, hexose-aminidase B, superoxide dismutase B, glutathione reductase, adenylate kinase-1, glutamic-oxaloacetic transaminase, lactate dehydrogenase A and B, nucleoside phosphorylase, pyruvate kinase, peptidase A, glucose phosphate isomerase, adenosine deaminase, superoxide dismutase A, and glucose-6-phosphate dehydrogenase. In no case was a correlation found between the presence of the human form of an enzyme in a hybrid and its susceptibility to HSV-1 infection, thus excluding by inference²³ a linkage to human chromosomes 1, 2, 5, 8, 9, 10, 11, 12, 14, 15, 18, 19, 20, 21 or X, respectively. Linkage to chromosome 17 is also excluded, since this chromosome carries the human thymidine kinase gene²⁴ which is required by all clones for survival in HAT medium.

Human chromosomes were readily distinguishable from those of the Chinese hamster in Giemsa-banded metaphase spreads of hybrid clones. Although extensive chromosome rearrangement was evident in one clone (PF4A1), in the majority of cases chromosome morphology was normal, and we were able to confirm the presence of those chromosomes whose presence was implied by the isozyme data. Of the remaining chromosomes only human chromosome 3 was present in all HSV-1 susceptible clones and absent from those which were non-susceptible. Although the total number of hybrid clones considered in this study is small the correlation shown in Table 4 is statistically significant ($\chi^2 = 6.20$; $0.01 < P < 0.05$), and we

therefore conclude that the gene(s) responsible for HSV-1 susceptibility in Chinese hamster \times human hybrids is located on human chromosome 3. The nature of the host factor(s) specified by this gene must remain the subject of further investigations.

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Influence of multiple syngeneic foetal heart grafting on individual graft survival

THE factors which influence many developmental phenomena are poorly understood. One would like to know, for example, how it happens that an organism only develops one heart. A possible clue to this particular question arose inadvertently from an immunological tolerance assay involving inter-strain grafting of murine foetal hearts into adult ear pouches¹. We noticed amongst the control syngeneic grafts that whereas single heart grafts had a high long-term survival rate, grafting two hearts into a single animal seemed to considerably reduce the individual graft survival rate. The experiments reported here extensively document this observation using different strains and different grafting sites, as well as control grafts for specificity.

The recipient animals used in the study were highly inbred CBA/J and BALB/Ccr males between 2 and 4 months of age and were obtained from our breeding facility at the University of Alberta. No animal of a given strain was more than four generations removed from the ancestral breeding pair generating any other animal of that strain. Pregnancies were timed by checking for the presence of vaginal plugs, and the day after plugging was labelled day 1. All grafts reported in the study were obtained from 17-d foetuses. The principal method of grafting consisted of preparing a pouch under the dermis of the ear with the aid of small sharp scissors and tucking the graft as deeply as

possible into the pouch. Vascularisation of the foetal heart in this position permits recording of the graft's electrical activity simultaneously with that of the adult heart by specialised electrocardiography¹.

The results of a large number of such grafts performed in two different strains and read over the course of a 10-week period are shown in Table 1. The data for the CBA/J strain indicate that grafts put singly into an animal have a much higher probability of functioning than do grafts placed in an animal along with a similar graft in the opposite ear. The effect is reasonably stable over 10 weeks and the differences between the groups for any individual week are quite apparent. For example, even at week 1 the difference is highly significant ($P < 0.0001$) when tested by the chi-square contingency method. The results with a different strain of mice, BALB/Ccr, are concordant, although the overall functioning of both single and double heart grafts is somewhat reduced. The differences, although not as striking, are significant and the effect seems to disappear with time due to reduced functioning of the single heart grafts. In addition, a third strain (CBA/CaJ) was tested using 14 single grafts and 14 double grafts and the results at 2 weeks are similar to the previous data (57% functioning single hearts and 21% functioning double hearts, $P < 0.05$).

One possible explanation for the reduced survival of double heart grafts is that the added time and trauma involved in grafting the second heart somehow compromise both grafts. In order to test this, hearts were grafted into the left ears and either 17-d foetal kidney or 17-d foetal testis explants were grafted into the right ears of a group of CBA/J recipients. The hearts were checked at 2 weeks by electrocardiographic activity and then the ears were sectioned to examine the survival of the foetal kidney and testis grafts. Table 2 shows that neither foetal kidney nor foetal testis significantly affected the functioning of the grafted hearts at 2 weeks post-transplantation. The effect, therefore, seems to be specific for foetal heart. In order to further rule out artefacts that might ensue from the site of grafting and the method of reading, heart grafts were placed under the kidney capsule and scored one week later for beating, using an operating microscope. A heart was scored as positive if any fasciculation whatsoever could be detected within 1 min of viewing. The single hearts were implanted in the left kidney capsule and the double grafts were implanted either in the same kidney capsule or the contralateral kidney capsule. Since neither of these two situations differed in terms of overall frequencies, the results have been pooled. Table 3 shows that grafting under the kidney capsule gives a slightly higher proportion of beating compared to grafting in the ear, but the difference still exists between single and double grafts in two different strains. In fact the relative proportions of functional grafts for the two strains are in agreement by both methods of grafting and assessment (Tables 1 and 3). Grafting three hearts has no greater effect on an individual graft's functioning than grafting two hearts.

Since the adult heart does not interfere with single heart graft survival it is reasonable to enquire what effect an established heart graft would have on a second heart graft implantation. Therefore, we grafted single hearts in the left kidney capsule and 1 or 2 weeks later grafted a second heart in the right kidney capsule. The results of such a delay are shown in Table 4. The second graft seems to have no influence on the functioning of the first graft. The second graft, however, shows a significantly lower proportion of beating ($P < 0.05$). The proportion of second grafts functioning is higher than two hearts grafted at the same time, but not significantly different. This indicates that whatever is mediating this effect will not influence a graft that has been established for a week or two. An established

Table 1 Heart grafts in ear pouches

	CBA/J				Week					
	1	2	3	4	5	6	7	8	9	10
Fraction double heart grafts functioning	0.49	0.57	0.60	0.59	0.58	0.57	0.60	0.57	0.59	0.59
Total number	102	122	116	116	98	98	94	82	64	64
Fraction single heart grafts functioning	0.94	0.86	0.86	0.84	0.86	0.86	0.81	0.80	0.82	0.82
Total number	65	64	64	64	64	63	63	59	45	45
Significance of difference, <i>P</i> less than	10 ⁻⁴	10 ⁻⁴	10 ⁻³	10 ⁻³	10 ⁻³	10 ⁻³	5 × 10 ⁻³	10 ⁻²	2 × 10 ⁻²	2 × 10 ⁻²
	BALB/Ccr									
Fraction double heart grafts functioning	0.40	0.40	0.41	0.42	0.39	0.34	0.36	0.34	0.39	0.41
Total number	146	146	126	96	96	76	76	76	56	56
Fraction single heart grafts functioning	0.58	0.54	0.58	0.57	0.53	0.55	0.55	0.53	0.45	0.34
Total number	103	103	103	108	68	53	53	53	38	38
Significance of difference, <i>P</i> less than	10 ⁻²	3 × 10 ⁻²	2 × 10 ⁻²	5 × 10 ⁻²	NS*	3 × 10 ⁻²	5 × 10 ⁻²	5 × 10 ⁻²	NS*	NS*

NS = Not significant (*P* > 0.05).

graft, however, can have some effect on a subsequent graft.

The data presented in this paper support the conclusion that the probability of an individual heart graft surviving in a syngeneic host is influenced by whether or not other hearts are grafted at the same time. This conclusion has been reached after examining a large number of grafts in two different strains and a smaller number in a third strain. There are a number of possible explanations for this observation. The first and most obvious one, that of observer bias, seems to be ruled out because the grafts were read blind using the electrocardiographic procedure. The results were extraordinarily consistent from one reading to the next. In other words, if a given animal had a positive left ear and negative right ear, this persisted from week to week during the period for which it was read, even though there was no way in which the reader could have known the previous results while the ears were being read. Also, at various stages three different people read the grafts and came to the same conclusion.

A second possible explanation could come from the additional time involved in grafting the second heart, which might compromise both grafts. In fact, the heart grafting can be done so rapidly with a little practice (approximately 30 s per ear) that this objection is highly unlikely. Furthermore, no adverse effect of grafting other foetal tissues into a contralateral ear was observed on single heart grafts.

Another explanation that needs to be considered is an immunological one. A segregating or foetal specific antigen might cause some of the heart grafts to be rejected. Three separate considerations render this explanation untenable. First of all, the difference between double and single heart grafts is apparent after 1 week (see Table 1). This would be too rapid for most types of graft rejection. Secondly, histological examination of nine electrocardiographically negative hearts removed 2 months after ear grafting revealed normal heart muscle tissue, indistinguishable from control positive grafts, and showing no sign of monocytic or polymorphonuclear infiltration. Finally, and most conclusively, the difference between double-grafted and single-grafted hearts persisted when the host animals were completely immunosuppressed. This was accomplished by irradiating CBA/J males with 650 R using a ¹³⁷Cs source

(Gamma Cell 40, Atomic Energy of Canada), a dose which completely suppressed the splenic plaque forming cell response to sheep red blood cells when immunised 24 h and assayed 5 d after irradiation. (Six unirradiated control immunised animals had a response of 8.41 (±3.91) × 10⁴ PFC per spleen. Six normal unimmunised mice had 153 (±190) PFC per spleen. Six mice given 650 R and immunised had 65 (±45) PFC per spleen.) The mice had heart grafts placed in their ears 24 h after irradiation and read electrocardiographically 7 d after grafting. Of the double grafts, 57% (8/14) were positive at one week, whereas 92% (11/12) of the single grafts were positive. This result closely resembles the data in Table 1 and indicates that complete immunosuppression of the host does not increase the percentage of functioning double heart grafts.

A more attractive explanation of these results might be the existence of a mechanism whereby a nascent heart sends out some signal to prevent another heart from developing along the same vascular tree. This could have obvious relevance for survival if the probability of more than one inductive signal for heart formation is appreciable. This speculation agrees with the experiment in which an established graft can influence the functioning of a graft put in subsequently, but the subsequent graft has no effect on the established graft. If some such mechanism exists and operates by a soluble factor it would not be possible for the factor to cross the placenta (at least in the maternal to foetal direction) because mice invariably have multiple offspring in the same litter. The reason for the existence of some doubly positive animals might be that the relatively large "space" of the adult host in the experimental situation (compared to the ordinary foetal "space") somewhat dilutes the effect.

Analogous observations have been made with grafted neonatal lymphoid organs. Metcalf showed that single neonatal spleen grafts placed subcutaneously into adult splenectomised mice grew much larger individually than did

Table 2 Specificity of double heart effect

Group	Left ear	Right ear	Fraction beating*†	Total number
1	Heart	—	0.86	64
2	Heart	Heart	0.57	122
3	Heart	Kidney	0.81	16
4	Heart	Testis	0.83	18

*Read at 2 week post-grafting.

†Groups 3 and 4 are not significantly different from group 1 (*P* > 0.9) but are significantly different from group 2 (*P* < 0.01).

Table 3 Heart grafts under kidney capsule

Strain	Multiplicity of graft	Fraction beating*	Total number
CBA/J	1	0.93	29
CBA/J	2	0.64	28
CBA/J	3	0.62	21
BALB/Ccr	1	0.64	12
BALB/Ccr	2	0.50	11

*Read at 1 week post-grafting.

Table 4 Effect of established graft on second graft

Fraction beating (total number) if second graft delayed for			
	1 week	2 weeks	Total
Established graft	1.00 (14)	0.93 (14)	0.96 (28)
Second graft	0.79 (14)	0.71 (14)	0.75 (28)

multiple grafts². In fact, the total spleen mass tended towards a constant value. Thus, six multiple grafts gave a total weight roughly equal to that observed with 12 multiple grafts. This effect was not observed when multiple spleen grafts were placed on the chorioallantoic membrane of chickens³. Metcalf found that neonatal thymus grafts, unlike spleen grafts, behaved autonomously, even though as many as 50 grafts were implanted subcutaneously, reaching a total weight of 800 g. Two different groups have reported nonetheless that the newborn offspring of neonatally-thymectomised females have enlarged thymuses compared to control litters born to sham-thymectomised females^{5,6}. The effect diminishes with subsequent pregnancies as the female herself becomes immunologically restored^{6,7}. It is not inconceivable, therefore, that a similar type of feedback mechanism operates during the development of the heart to prevent the disastrous consequences of two autonomous pumps operating on the same vascular tree.

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Myelin deficit in the Jimpy mouse may be due to cellular abnormalities in astroglia

THE mouse mutant Jimpy is considered to be a model for certain human diseases in which the development of myelin is abnormal^{1,2}. Biochemical studies of Jimpy have shown that myelin components are reduced but no specific abnormality in the assembly of myelin has been detected²⁻⁴. This electron microscopic study describes for the first time abnormalities in astroglia of Jimpy optic nerve that are likely to inhibit the development of oligodendroglial cells and myelination.

Before the onset of oligodendrocyte formation in these mutants, processes of astrocytes begin to branch abnormally. The branching continues until most axons are surrounded by astrocytic processes. Such an arrangement would make it necessary for the oligodendrocytes to displace the glial processes in order to contact axons and make compact myelin. The cause of the astrocytic branching in Jimpy has not been proven but it may be related to the paucity of microtubules and profusion of 90-Å filaments found in astrocytes during the early stages of development. Microtubules are necessary for maintaining cell shape^{5,6}, so it is possible that their paucity and/or the filamentous hyperplasia is the underlying morphological defect responsible for the shape of astroglia in Jimpy.

Jimpy mice, obtained from breeding colonies sent by Jackson Laboratories, were studied at 2, 5, 10 and 23 d after birth. Optic nerves were examined at all stages and compared with spinal cords at 2 and 23 d after birth. Female hemizygotes were initially used as the only controls but

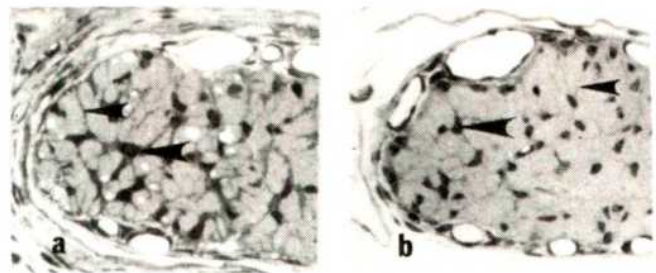


Fig. 1 Transverse sections of optic nerves from a 2-d postnatal Jimpy mouse (a) and a hemizygote (b). Both pictures are at the same magnification ($\times 150$) and they illustrate differences in the size of nuclei (large arrows) and the hypertrophy of astroglial processes (small arrows). The degree of the hypertrophy in Jimpy optic nerves is variable from animal to animal and even within the nerve. Note that the left side seems more affected than the right. The vacuoles are not artefacts of fixation but seem to be swollen, degenerating axons at the fine structural level.

these animals showed probable glial and axonal abnormalities at the fine structural level. Accordingly, homozygous normals and Swiss mice were also used.

During early postnatal development in the optic nerves of normal mice and rats, astrocytic processes divide the neurites into distinct fascicles⁷⁻⁹. These processes do not have many side branches but in Jimpy many fine branches penetrate into the axonal fascicles isolating neurites from each other (Fig. 2). This hyperplasia of astrocytic processes continues during myelinogenesis^{10,11} until the majority of axons are surrounded (Fig. 3).

In normal animals at 2 d after birth, the cytoplasm of astroglial cells contains an abundance of organelles including microtubules; the clusters of filaments characteristic of mature forms are less common⁷⁻⁹. In Jimpy mice at 2 d after birth, this relationship is reversed so that there is a paucity of microtubules and an abundance of filaments (Table 1). There is almost a fivefold difference in the number of microtubules in Jimpy when compared to Swiss

Fig. 2 Electron micrograph of the optic nerve from a 2-d-old Jimpy animal showing the branching of astrocytic processes (Ap) into fascicles of axons (A). The large, radially oriented processes give off numerous small branches which divide the axons into small compartments. Some of these processes have begun to separate individual axons from other axons (arrows). This extensive branching does not occur in normal animals (see references 7-9 for comparison illustrations). The radially oriented astroglial processes contain primarily 90-100-Å filaments but few microtubules (Table 1) ($\times 8,800$).



Table 1 Comparison of number of microtubules and filaments in astroglia of 2-d postnatal optic nerve

	Swiss strain	Hemizygote	Jimpy
No. of astrocyte microtubules per μm^2 of astrocyte cytoplasm	34.3	16.0	7.2
No. of astrocyte microtubules per μm^2 of optic nerve tissue	7.5	4.3	2.2
No. of astrocyte 90-Å filaments per μm^2 of astrocyte cytoplasm	~480*	~640	~1170
Ratio of astrocyte cytoplasm to optic nerve tissue	1:4.6	1:3.9	1:3.3

The number of longitudinally sectioned microtubules in astroglia was counted from electron micrographs printed at 13,000 and viewed with a Bausch and Lomb $\times 7$ magnifier. Ten to fifteen random pictures were used for each optic nerve; half of the pictures were taken from the centre of the nerve and the other half at the periphery. The area of the astrocyte cytoplasm was calculated with a Lasico planimeter. The area of optic nerve tissue included all cell nuclei and processes with the exception of vascular elements. The area of astrocyte cytoplasm surveyed ranged from 6.5 to 7.3 μm^2 . The quality of fixation for all animals was approximately the same.

* The precise number of 90-Å filaments is impossible to determine because the filaments overlap each other and weave in and out of the plane of the picture. The estimate for Jimpy is probably low because of the dense packing of filaments within a cluster.

mice and a twofold difference for the hemizygotes. It might be argued that the microtubule deficit is only an apparent one because the astrocytic cytoplasm is hypertrophied. But when the number of microtubules in astroglia is compared to the area of optic nerve (Table 1), there is still a two- to fourfold difference between Jimpy and the controls. The difference in the ratios between astrocytic cytoplasm and optic nerve area confirms the light and electron microscopic observations that there is a hypertrophy of astrocyte cytoplasm (Fig. 1). In contrast to the microtubule deficit, the number of 90-Å astroglial filaments is increased more than twofold (Table 1). Electron microscopic observations show that not only are the clusters of filaments more numerous but also that the filaments within the clusters are more densely packed. This observation correlates well with an immunofluorescent study¹² of astroglial fibrillary acidic protein in Jimpy mice. Although not commented on by these authors, the fluorescence for this substance is greater in Jimpy spinal cords than in the littermates used as controls (Fig. 1). This study shows that microtubule and filament abnormalities occur to a lesser degree in the hemizygotes.

While the astroglial cells in Jimpy optic nerve are strikingly abnormal in appearance, most of the oligodendroglia seem normal except that they are less differentiated than the controls. The number of oligodendroglial cells is, however, reduced more than 50% (Table 2). The percentage reduction of oligodendroglia depends, to some extent, on the interpretation of the unclassifiable cells. If all of the unclassifiable cells are considered to be oligodendroglia, there is a 48% reduction at 10 and 23 d after birth; if none is considered oligodendroglia, the loss is 61% and 79%. But approximately half of the unclassified cells at 10 and 23 d after birth seem to be oligodendrocytes and using this estimate, there is about a 53% and 62% reduction. An apparent reduction in the number of oligodendroglia in the corpus callosum has been noted^{13,14} but the severity of this deficit was not quantitated.

In previous studies of Jimpy nervous system, investigators have focused upon the period of myelin formation, and have primarily confined themselves to possible oligodendroglial and myelin abnormalities^{2-4,10,13-15}. The hyperplasia of astroglial processes described here for the optic nerve is, however, frequently noticeable in the published pictures (ref. 15, Figs 7 and 8). We show that these abnormalities are apparent at least a day before oligodendroglia are present and 3 or 4 d before myelination begins^{10,11}. This observation indicates that the astroglial hyperplasia is not triggered by the presence of oligodendrocytes.

The astroglial abnormalities raise the interesting question as to how they can affect the oligodendroglial cells. It is conceivable that the hyperplasia of astroglial processes, which occurs before and during myelinogenesis, could block the oligodendrocytic processes from reaching axons and forming proper internodes. However, it is more difficult to explain how the proliferation of oligodendrocytes can be



Fig. 3 Electron micrograph of a Jimpy optic nerve 23 d after birth. The astrocytic processes (Ap), most of which have been outlined, now surround the majority of axons (A). The glial processes are identified by the presence of numerous 90–100 Å filaments which appear as clusters of dots at this magnification. The profile of an oligodendrocyte (Ol) is identified by its electron dense cytoplasm and nucleus. The clumping of chromatin beneath the nuclear membrane is also a characteristic feature of this cell type. Three axons are surrounded by four to six turns of myelin lamellae ($\times 14,500$).

affected. Although the factors controlling oligodendroglial proliferation are unknown, the proliferation of the myelin-forming cells in the peripheral nervous system is stimulated by the presence of axons¹⁶. Perhaps the astroglial hyperplasia impedes contact between the axons and oligodendrocytes. Another possibility is that the normal number of oligodendroglial cells is generated but that some die shortly after cell division, due to lack of contact with axons. Thymidine autoradiographic studies should provide information about the proliferation kinetics of these cells. A third possibility is that the source of the oligodendrocytes might be affected. The origin of the oligodendroblast stem cell is

Table 2 Ultrastructural classification of different cell types present in a complete transverse section of optic nerve.

	Astroglia	Oligodendroglia	Microglia	Unclassifiable	Total cell no.
2-d postnatal Swiss mouse	54	0	0	19	73
2-d postnatal hemizygote	57	0	0	15	72
2-d postnatal Jimpy*	61	0	2	3	66
10-d postnatal homozygous normal	70	81	1	28	180
10-d postnatal Jimpy	75	32	1	25	133
23-d postnatal homozygous normal†	59	97	4	12	172
23-d postnatal Jimpy	60	20	8	37	125

The ultrastructural criteria for classification of differentiating glia in optic nerve is discussed in detail by Skoff *et al.*⁷ In the present study, two characteristic features of each cell type were required for a definitive classification (for example, filaments and electronluculent appearance for astrocytes). It is difficult to classify precisely all of the cells because some do not have sufficient characteristics for a positive identification or they have been sectioned through areas lacking cytoplasm. Complete transverse sections of the optic nerve were mounted on Formvar coated slot grids for electron microscopic examination. The cell counts were made directly from the viewing screen of an AEI-801 electron microscope. A scanning system was devised to avoid recounting the same cell; low magnification montages were made of two sections to check possible errors in counting. There was a 1 and 3% difference in the number of labelled cells using the scanning and montage methods. Only one animal was used for quantitation but at least two Jimpy animals were examined at each stage except at 23 d after birth when only one animal was available. The procedure for preparing optic nerve tissues for electron microscopy has been presented elsewhere⁷.

*2 d postnatal animals were identified as Jimpy on the basis of differences in the amount of myelin present in the spinal cord. At this time, axons in the ventral funiculus are beginning to myelinate and the spinal cords of Jimpy animals show fewer myelinated axons.

†The number of cells counted in a transverse section of a homozygous normal (172) compares favourably with a light microscopic cell count (162) by Friedrich¹⁸ of a (C57BL/6J) mouse.

uncertain but there are two likely sources⁷, a population of undifferentiated cells or the poorly differentiated astroblasts. The number of undifferentiated cells and poorly differentiated astroblasts is reduced in Jimpy optic nerve 2 d after birth, in comparison to controls.

Previous morphological and biochemical studies of Jimpy mice have shown that the scarcity of myelin is restricted to the central nervous system¹ and that the deficit is not due to a demyelinating disorder^{2,18}. Up to the present, however, no specific abnormality in the formation of myelin has been detected by biochemical studies²⁻⁴. The reduction in the number of oligodendrocytes described here is certainly one of the major factors responsible for the paucity of myelin in Jimpy but it does not elucidate the cause of the cellular reduction nor does it explain certain abnormalities in the appearance of the few myelinated fibres. The hyperplasia of astrocytic processes, which begins before the formation of oligodendroglia, could account for the myelin and oligodendroglial abnormalities. The hypothesis that astroglia affect oligodendrocytes is new but a precedent for such a cellular interaction in the developing central nervous system occurs between astrocytes and neurones in which the migrating neuroblasts fail to reach their destination in the absence of astroglial processes¹⁷. The effect of an overproduction of astrocytic processes on neuronal development has yet to be determined but the precocity of the "gliosis" in Jimpy, occurring when axons are still growing through the optic nerve, may be a model for studying the interactions between astroglia and axons in development.

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Reversible loss of hippocampal long term potentiation following electroconvulsive seizures

ONE of the most intriguing electrophysiological phenomena observed in the central nervous system is the persistent enhancement of an evoked synaptic response following brief trains of electrical stimuli. Within the hippocampus, evoked synaptic potentials can be doubled or tripled by tetanising stimulation, and such changes persist for hours to weeks¹⁻⁶. Interest in the phenomenon of long term potentiation (LTP) stems from the generally held belief that synaptic plasticity of this sort may well be a physiological basis of information storage in the CNS. It is notable that this striking synaptic plasticity is observed in a structure which extensive clinical and experimental evidence suggests has a central role in information storage and/or retrieval⁷⁻¹¹. Included in this evidence are numerous demonstrations that disrupting hippocampal activity by electrically induced epileptiform seizures also disrupts memory formation and/or retrieval¹²⁻¹⁴. In addition, the effectiveness of a variety of other treatments in disrupting behavioural plasticity seems closely related to their ability to elicit convulsive activity in the hippocampus^{15,17}. We report here a series of experiments indicating that LTP established in the hippocampus can also be reversibly disrupted by the same kind of convulsive activity which disrupts memory functions. Specifically, we have produced LTP in the CA1 pyramidal cell synaptic field, disrupted it with electrically induced hippocampal seizures, and subsequently reinstated it with low frequency tetanic stimulation.

Adult Lashley rats were given atropine sulphate (intramuscularly, 0.2 mg kg⁻¹) and anaesthetised with an intraperitoneal injection of allobarbitol (60 mg kg⁻¹) and urethane (240 mg kg⁻¹). The hippocampus was exposed by aspirating the overlying cortex, and was continuously bathed in warm (37-38 °C) mineral oil. Electrodes were placed to stimulate fibres within the stratum radiatum of the CA3 field and to record the extracellular population excitatory postsynaptic potential (e.p.s.p.) from stratum radiatum of the CA1 field. An additional electrode, well removed from the other two, was used to generate electroconvulsive

seizures in the hippocampus. In most cases an input-output function was first obtained by recording the CA1 synaptic response to CA3 stimulation over a range of stimulus intensities. Subsequently, several low frequency trains of impulses (12 Hz for 15 s, 8 min inter-train interval) were delivered while monitoring the CA1 synaptic response with a probe stimulus continually applied at 0.2 Hz.

The low frequency tetanic (LFT) stimulation produced long term increases in the evoked population e.p.s.p. amplitude ranging up to about 300% (mean 175.8%), along with decreases in the stimulus intensity required to elicit a population spike from the CA1 pyramidal cells. The effects of LFT stimulation were cumulative, and maximum potentiation was usually obtained after three to six LFT trains. That the potentiation was truly long term was established in several experiments where it persisted with little or no decline for periods of over an hour (the duration of the experiment) following the last LFT.

In 18 experiments hippocampal seizures were induced at various intervals following a series of LFT trains. Seizures were evidenced by afterdischarges lasting up to 45 s, followed by complete post-ictal depression of the response for up to 10 min, then a period of gradual response recovery. Recovery was considered complete when the response showed no further change for 10 min. The overall recovery periods ranged from 15 to 25 min following the seizure.

The effect of these induced seizures was dramatic (Fig. 1). In all 18 experiments the LTP established by repeated tetanic stimulation was wholly or partially abolished. In none of these experiments did the post-seizure response recover to the potentiated response level, and in all but two cases the post-seizure response recovered to at least the level of the initial unpotentiated control response. In both of these cases a sudden drop in the response during the re-

covery period suggested probable electrode movement. The magnitude of the drop was approximately equal to the difference between the final recovery and initial control responses. Excluding these two experiments, the effect of the seizures was to reduce the LFT-induced response potentiation by a mean 83.5% (the mean post-recovery response was 119.8% of the initial control response).

The interference with LTP produced by the seizure seemed partially or wholly reversible. In ten experiments additional LFT trains were administered after a seizure had wholly or partially eliminated the LTP established by the initial LFT stimulation. In eight of these experiments LTP was re-established, and such plasticity was evident through as many as four cycles of the LFT-seizure-recovery procedure (Fig. 2).

As is evident in both Figs 1 and 2 there was, in general, a progressive decrease in the amount of LTP which could be realised following successive seizures. In those experiments in which LFT was administered after a seizure, the second LFT series produced only about half (45.2%) as much plasticity as the pre-seizure stimulation produced. Comparable decreases in plasticity seemed to be the rule following a second or third seizure. It is not clear whether the decreasing potential for plasticity represents a real limitation of the processes involved or is simply a "tired tissue" phenomenon. The latter possibility seems probable, since it is noted that less electrophysiological plasticity of any type is evident in longer experiments³.

These results might be interpreted as being due to an inadequate period of recovery following the seizure. To test this possibility, post-seizure responses were monitored in several experiments for 20–50 min after stabilisation rather than the usual 10 min (Fig. 2c). Despite this, no additional recovery was noted. Hence, these results cannot

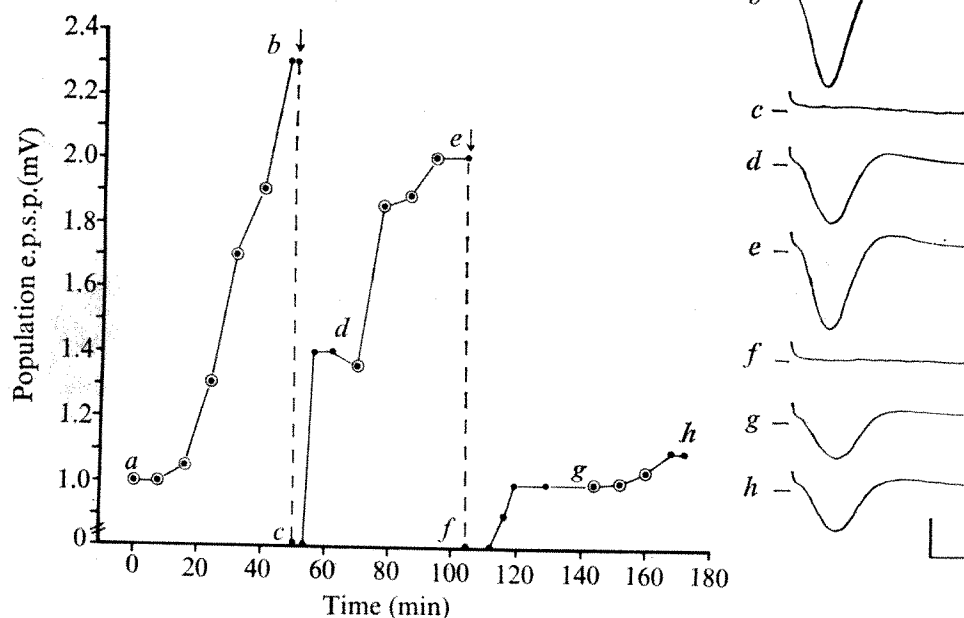


Fig. 1 CA1 extracellular population e.p.s.p. response magnitude to test stimuli during the establishment of long term potentiation (circled points), its interruption by electrically induced electroconvulsive seizure (arrows), and recovery from seizure related post-ictal depression (following the dashed lines) in a typical experiment. The circled points are test responses immediately preceding low frequency tetanic stimulation (12 Hz for 15 s). The low frequency tetanic stimulation resulted in increases in synaptic efficacy which were abolished by the seizure. Following recovery from post-ictal depression, additional tetani again resulted in a potentiated response, but of progressively lower magnitude. Raw records are shown corresponding to the indicated points on the graph. CA1: 1 mV, 5 ms. Control (0.2 Hz, 0.1 ms duration, 5–20 V) and tetanic (12 Hz, 0.1 ms duration, 5–20 V) stimulus pulses were delivered through an insulated monopolar stainless steel electrode (20–40 μ m exposed tip) placed in stratum radiatum of CA3 to activate axons (primarily Schaffer collaterals) projecting to CA1. A similar monopolar recording electrode was placed in stratum radiatum of CA1 (about 4 mm posterior to the stimulating electrode) to record the CA1 extracellular population e.p.s.p. A similar monopolar stimulating electrode was placed 1 mm deep posterior and lateral to the recording site. Seizures were induced through this electrode by a 1-s train of 60 Hz, 15 V pulses (biphasic, 1 ms duration, roughly 300–400 μ A).

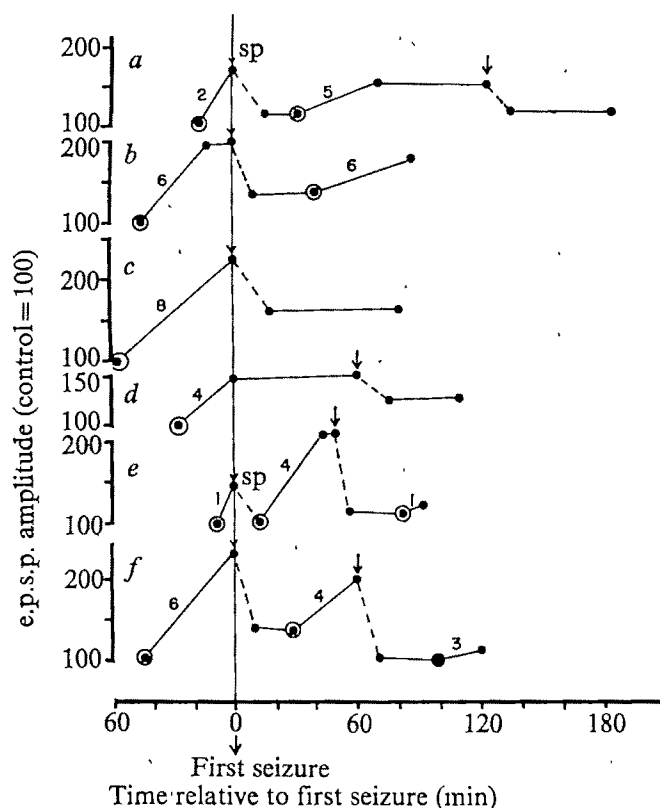


Fig. 2 Six experiments (a-f) in which a number of variables were manipulated relative to seizure disruption of long term potentiation. Circled points are extracellular population e.p.s.p. magnitudes plotted relative to a pre-tetanus control (= 100). The numbers refer to the number of tetanic stimulations delivered; the following point is the stimulation induced potentiation. Electrically-induced or spontaneous (sp) seizures are indicated by arrows. The first seizure occurs at time zero (except for d). Note that the seizure interferes with long term potentiation regardless of the number of tetani, time after initial potentiation, or means of inducing seizures. Additional tetani result in repotentiation.

be readily attributed to inadequate recovery from seizure.

Another alternative explanation for these results is that the electroconvulsive stimulation and seizures produced a generalised decrease in the excitability of the hippocampus. That this was not the case is evidenced by several types of data. Seizure and recovery prior to LFT stimulation did not alter the response to the probe stimulus, nor did this preclude subsequent establishment of LTP. In some experiments where no LTP was observed, a seizure was nevertheless induced following several ineffective LFT trains. In these cases the post-recovery response was virtually unchanged from the pre-seizure level. In addition, in only two experiments (those noted earlier) was the threshold stimulation required to elicit a population e.p.s.p. appreciably different following seizure recovery than the threshold before the seizure, and before the LFT stimulation¹⁴. It should also be noted that in most cases pre-seizure LFT stimulation was continued until no further response increases were observed, and the response was "fully potentiated." In spite of this, additional plasticity was evident following a seizure. Finally, in three cases "spontaneous" seizures occurred during an experiment with the same result as electrically-induced seizures—total or partial elimination of LTP (Fig. 2a, e).

The data presented here indicate that electroconvulsive seizures induced in the hippocampus interfere with the processes underlying long term potentiation of evoked synaptic responses, and that this interference is at least partially, and perhaps wholly, reversible. Thus, hippocampal seizures seem to have quite analogous effects on both behavioural and electrophysiological plasticity. The evidence presented

here does not allow a decision on the relative importance of the convulsive activity *per se* against that of the post-ictal spreading depression (SD) in producing these effects. However, preliminary observations of a similar effect following topical application of KCl suggest that SD is the essential correlate of LTP interference.

We cannot comment conclusively on mechanisms which might mediate the effects described here, since the mechanisms underlying LTP are as yet unclear. Nor do we know if processes similar to these occur as a normal concomitant of hippocampal functioning. But our results seem consistent with one proposed mechanism of LTP. Van Harreveld and Fikova¹⁸ have proposed that LTP is the result of stimulation-induced swelling, and consequent conduction increase, of dendritic spines. Anatomical evidence seems to support their assertion, and there is limited evidence of the possible behavioural relevance of this mechanism^{19,20}. It is also notable that SD is accompanied by dendritic and somatic swelling, presumably mediated by the same mechanism responsible for stimulation-induced spine swelling, though in a more extreme manifestation. Since SD swelling is clearly reversible²¹, it is plausible to suggest that the extensive SD induced by seizures in our experiments triggered recovery processes which reversed not only dendritic and somatic swelling, but the presumed LFT-induced spine swelling as well. Thus, recovery from a seizure would be accompanied by a reduction or elimination of any LTP existing prior to the seizure, but would not preclude the re-establishment of LTP by further stimulation. Though this is clearly only a tentative hypothesis, it is consistent with the available data, and it does suggest additional experiments which could decide these issues.

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Evidence for dopamine receptors mediating sedation in the mouse brain

APOMORPHINE, a direct stimulant of dopamine (DA) receptors, and L-dopa, the direct precursor of DA, have a biphasic action on behaviour, in low doses they decrease motor activity, while in higher doses they cause hypermotility and stereotypy¹⁻³.

While the stimulant effect is considered to be due to the activation of postsynaptic DA receptors in brain^{4,5}, it has been suggested that the depressant response to these drugs is due to the activation of DA "autoreceptors", which would

result in inhibition of DA synthesis and dopaminergic firing^{3,6,7}.

However, while there is experimental evidence that the inhibition of DA synthesis and neuronal firing, which is blocked by neuroleptics, is due to stimulation of DA receptors^{8,9}, no such evidence exists for the sedative effect. Moreover the idea that the sedative effect of apomorphine depends on decreased dopaminergic activity is simply based on the observation that the two events are correlated in dose-response studies².

The demonstration that the sedative response to apomorphine and L-dopa is due to stimulation of DA autoreceptors is hampered by the fact that neuroleptics, which are antagonists at CNS DA receptors, themselves cause sedation.

We considered that some neuroleptic compounds might be found with higher affinity for auto- than for postsynaptic receptors and that such compounds might thus demonstrate the DA-mimetic nature of the sedative effect of DA receptor agonists.

We report here that some neuroleptics, at doses lower than those producing sedation, are capable of preventing the sedative effect of apomorphine and L-dopa in mice. At these doses these compounds are also effective in preventing the apomorphine-induced decrease in DA synthesis, as reflected by brain DA metabolite levels.

Figure 1 shows the effect of 0.1 mg kg⁻¹ of subcutaneous apomorphine in control mice and in mice pretreated with pimozide at the non-sedative dose of 0.3 mg kg⁻¹, 20 min previously.

Apomorphine decreased motor activity, and this effect

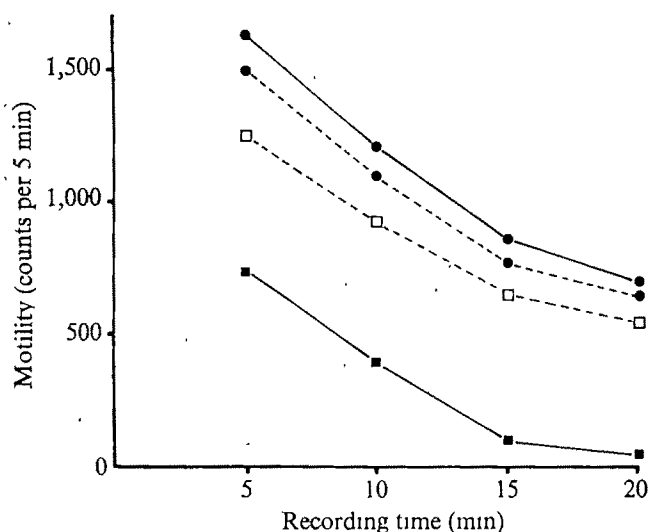


Fig. 1 Effect of pimozide on the hypomotility produced by apomorphine in mice. Mice were pretreated with pimozide (0.3 mg kg⁻¹ intraperitoneally) 20 min before apomorphine (0.1 mg kg⁻¹ subcutaneously). Ten min after apomorphine the animals were placed for the first time in the motility cage (Motron, Sweden) and motility was measured immediately. In these conditions, this generally took the form of exploratory activity. At the dose used in this experiment, apomorphine dramatically reduced the activity such that after the first 10 min, while control mice were still highly active, mice treated with apomorphine lay down in a corner and appeared sedated. The schedule used for pimozide was the most effective in preventing the sedation produced by apomorphine without producing sedation *per se*. Use of longer time intervals (3 h) for pretreatment with the 0.3 mg kg⁻¹ dose of pimozide results in sedation. However, similar antagonism of apomorphine hypomotility, even if less dramatic, was obtained after a 3-h pretreatment with 0.1 mg kg⁻¹ of pimozide. The results are the mean of four experiments. Open symbols indicate a significant difference ($P < 0.01$) between the curves with the same symbols as evaluated by non-parametric multivariate analysis¹⁰. ■, apomorphine; □, pimozide and apomorphine; ○, pimozide; ●, control.

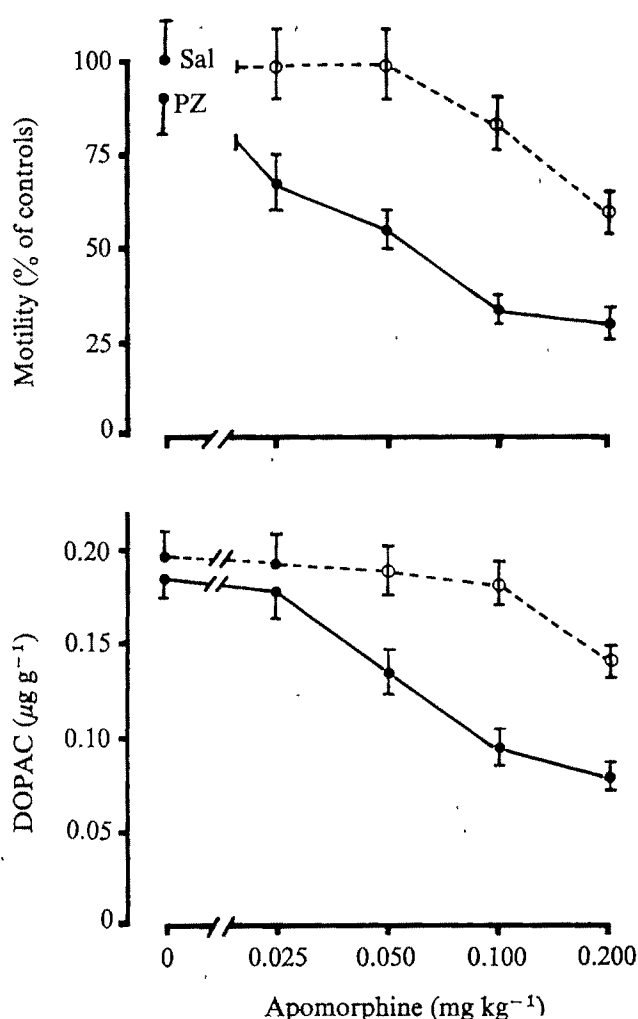


Fig. 2 Antagonism by pimozide of the hypomotility and decrease of brain DOPAC produced by apomorphine in mice. Pimozide (0.3 mg kg⁻¹ intraperitoneally) was administered 20 min before apomorphine. Apomorphine was administered subcutaneously and 10 min later the animals (4 cage) were placed in the motility meters (Motron, Sweden), and motility counted for 20 min. Mice were killed at the end of the motility test and DOPAC assayed fluorometrically according to Di Chiara *et al.* (unpublished). The effect of pimozide on apomorphine hypomotility was calculated as % of the motility obtained after pimozide alone. The results are the mean \pm s.e.m. of 4 experiments. Open circles indicate a significant difference ($P < 0.01$) with respect to apomorphine alone. ●, apomorphine; ○, pimozide and apomorphine.

was prevented by pimozide; the latter also prevented apomorphine-induced decrease of brain 3,4-dihydroxyphenyl acetic acid (DOPAC), the deaminated metabolite of DA.

Figure 2 shows the effect of increasing doses of apomorphine on the motor activity and brain DOPAC of control mice and of mice pretreated with pimozide, 0.3 mg kg⁻¹, given 20 min earlier. Apomorphine caused a dose-dependent decrease in motor activity and of DOPAC levels both in control and in pimozide-treated mice. However, pimozide, which *per se* did not significantly influence motor activity or brain DOPAC, shifted to the right the dose-response curve of apomorphine-induced hypomotility and brain DOPAC decrease, suggestive of competitive antagonism.

The antagonism exerted by pimozide on the effects of apomorphine was not related to a modification of the tissue distribution of apomorphine, since pimozide did not modify the time-course of the levels of ³H-apomorphine in brain (results not shown).

Similar results were obtained with other neuroleptics such as haloperidol, benzperidol, droperidol and sulpiride: as shown in Table 1, these compounds, at doses which did not

influence motor activity or brain DOPAC, prevented both the hypomotility and the fall in brain DOPAC produced by apomorphine.

On the contrary, clozapine and trifluoperidol, at non-sedative doses, failed to influence either the sedative or the biochemical effects of apomorphine.

Neuroleptics active against the inhibitory effects of apomorphine were also able to prevent the sedative effect of L-dopa, when administered at a schedule similar to that used against apomorphine. As Fig. 3 shows, sulpiride shifted to the right the dose-response curve of L-dopa-induced hypomotility, again suggesting a competitive-type antagonism, as for pimoizide against apomorphine.

In the above conditions, sulpiride did not influence the amount of DA formed in brain after L-dopa administration.

The present results show that different neuroleptics, such as haloperidol, droperidol, pimoizide, benzperidol and sulpiride are able to prevent the sedative effect of apomorphine and L-dopa and also the ability of apomorphine to inhibit the activity of the DA system, as estimated by the decrease in brain DOPAC^{8,10}. Since neuroleptics are specific and competitive antagonists of the DA receptors both *in vivo* and *in vitro*¹¹⁻¹³, the present results strongly indicate that apomorphine and L-dopa produce sedation and decrease dopaminergic activity by stimulating central DA receptors, as suggested by Carlsson and by Strömbom^{2,3}. Moreover, the strict correlation between the behavioural and biochemical changes indicate that the sedative effect of apomorphine depends on the decreased DA activity.

As to the nature of the DA receptors mediating the inhibitory effects of L-dopa and apomorphine, they should be considered quite distinct from the inhibitory receptors

Table 1 Effect of various neuroleptics on the hypomotility and brain DOPAC decrease produced by apomorphine

Neuroleptic	Dose (mg kg ⁻¹)	Motility (% of control)	DOPAC (μg g ⁻¹)
Control	—	100 ± 11	0.185 ± 0.008
None	—	25 ± 3.2	0.095 ± 0.006
Benzperidol	0.050	85 ± 9.5	0.180 ± 0.010
Droperidol	0.050	80 ± 7.5	0.178 ± 0.009
Haloperidol	0.050	75 ± 8.5	0.175 ± 0.008
Pimoizide	0.300	83 ± 9.3	0.182 ± 0.009
Sulpiride	10.0	87 ± 8.5	0.192 ± 0.010
Trifluoperidol	0.050	27 ± 3.0	0.098 ± 0.007
Clozapine	0.50	23 ± 2.5	0.097 ± 0.006

All neuroleptics were administered intraperitoneally 20 min before apomorphine (0.1 mg kg⁻¹ subcutaneously); mice were placed in the motility cages (4 mice per cage) 10 min after apomorphine administration and motility counted for 20 min. At the end of the motility test, the animals were killed and DOPAC assayed fluorimetrically according to Di Chiara *et al.* (in preparation). The dose of neuroleptic reported in the table is the lowest non-sedative one which was found experimentally to be maximally effective in antagonising apomorphine hypomotility. In the case of trifluoperidol and clozapine, for which antagonism could not be shown, the dose reported is the highest non-sedative one. At the dose reported, neuroleptics alone did not modify significantly the control levels of brain DOPAC nor the motility. Nonetheless, the effect of neuroleptics on apomorphine hypomotility was always calculated as % of the motility obtained when the neuroleptics were administered alone.

Each value is the mean ± s.e.m. of 4 experiments. All neuroleptics, except trifluoperidol and clozapine, significantly ($P < 0.01$) modified motility and brain DOPAC with respect to apomorphine alone.

postulated by Cools *et al.*¹⁴, which are not stimulated by apomorphine and are not blocked by neuroleptics. Our results support the concept of regulatory DA receptors by which the DA system controls its own activity (autoreceptors)³. The exact location of these receptors is not clear. They might be prejunctional, that is, located on the membrane of the DA neurone itself and stimulated by an overflow of the transmitter¹⁵, or in the substantia nigra, where a postsynaptic DA-sensitive adenylate cyclase^{16,17} and a vesicular release of DA from dendrites have been shown¹⁸.

The results reported here indicate the possibility of influencing behaviour by acting with drugs on a special type of DA receptor; this might constitute the basis for the development of more potent and selective blockers of DA autoreceptors and might provide an entirely new means of controlling the activity of the DA system.

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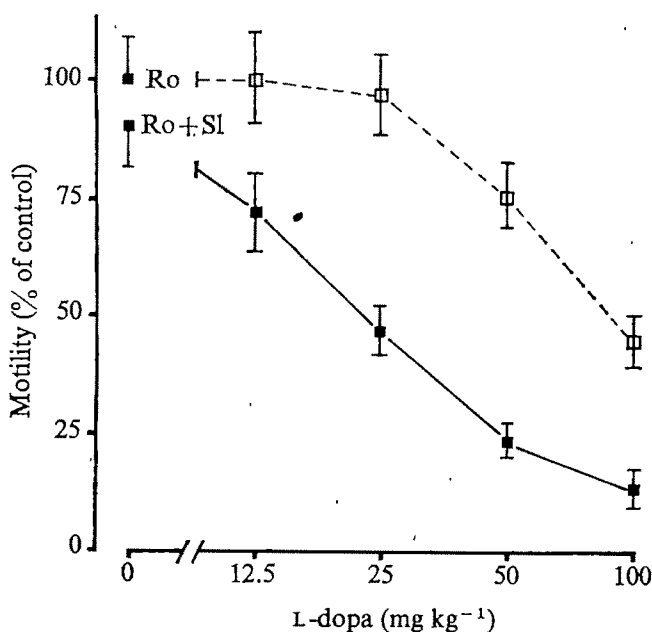
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Fig. 3 Antagonism by sulpiride of the hypomotility produced by L-dopa in mice. Mice were pretreated with 10 mg kg⁻¹ intraperitoneally of sulpiride, and 10 min later various doses of L-dopa were administered intraperitoneally together with a standard dose of the decarboxylase inhibitor Ro 4-4602/1 (50 mg kg⁻¹). Control mice received Ro 4-4602/1 alone or after sulpiride. Mice were placed in the motility cages 30 min after L-dopa+Ro 4-4602/1 and motility counted for 20 min. The effect of sulpiride on L-dopa hypomotility was calculated as % of the motility obtained after sulpiride+Ro 4-4602/1. The results are the mean ± s.e.m. of 4 experiments. Open symbols indicate a significant difference ($P < 0.01$) in respect to L-dopa+Ro 4-4602/1 □, sulpiride+L-dopa+Ro; ■, Ro+L-dopa.



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β -Endorphin *in vitro* inhibition of striatal dopamine release

THERE has been considerable recent interest in endogenous peptides as possible analgesic agents since the isolation of a pentapeptide with opiate-like activity (methionine-enkephalin) from porcine brain¹. Of particular interest is β -endorphin, isolated from camel pituitary glands², and now synthesised³. We report here the inhibitory effect *in vitro* of β -endorphin on striatal dopamine release from the central nervous system (CNS).

β -Endorphin consists of 31 amino acid residues with a sequence identical to amino acid residues 61-91 in the 91 amino acid peptide of ovine β -lipotropin^{4,5}. The first five amino acids of β -endorphin (residues 61-65 in β -lipotropin) are identical with methionine-enkephalin (Met⁵-enkephalin)¹ and were found to possess opiate activity in receptor binding assays and in electrically stimulated preparations of mouse vas deferens and guinea pig ileum. Subsequent studies have shown that β -endorphin also possesses opiate activity in receptor binding assays and in guinea pig ileum^{6,7}. More recently, it has been found that β -endorphin, in contrast to Met⁵-enkephalin, has potent analgesic properties when administered directly into the brain and assessed in the tail-flick, hotplate, and writhing tests in mice and in the wet shake test in rats⁸. In these tests, β -endorphin was found to be 18-33 times as potent as morphine on a molar basis and its actions were inhibited by the opiate antagonist naloxone. Although also antagonised by naloxone, Met⁵-enkephalin is an extremely weak and short-acting analgesic peptide⁹. In addition, β -endorphin was shown to be 3-4 times more potent than morphine when injected intravenously¹⁰.

The purpose of this study was to compare the abilities of morphine, β -endorphin and Met⁵-enkephalin to inhibit the

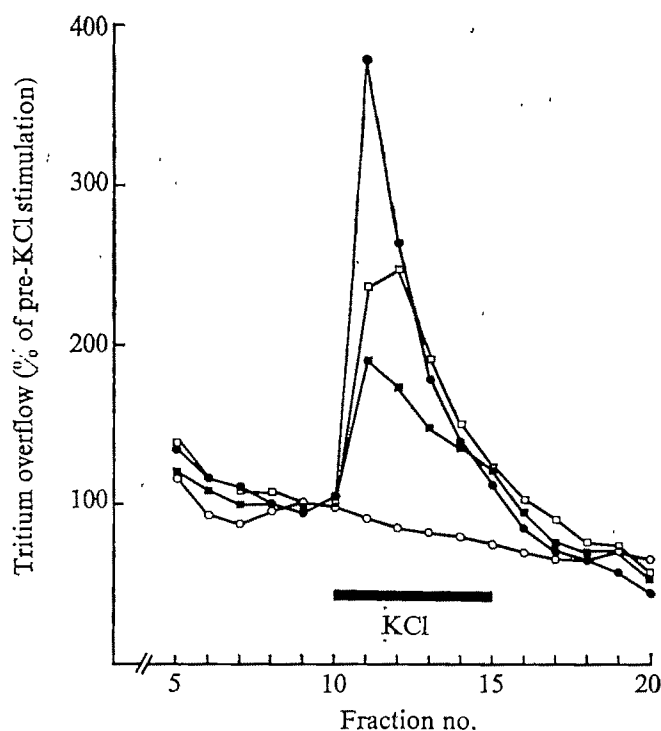


Fig. 1 Concentration-dependent inhibition of potassium-stimulated tritium overflow by β -endorphin. Concentrations of β -endorphin are indicated as follows: 0 μ M (●—●); 0.6 μ M (□—□); 1.2 μ M (■—■); and 3.0 μ M (○—○). Male Sprague-Dawley rats (200-350 g) were administered the monoamine oxidase inhibitor nialamide (100 mg kg⁻¹ intraperitoneally) 1 h before they were killed. Striatal tissue was dissected from a coronal slice of rat brain (1.5 mm thick) and incubated at 37°C in oxygenated (95% O₂, 5% CO₂) Krebs-Ringer bicarbonate (KRB) buffer (pH 7.4) containing ascorbic acid and 10⁻⁷ M ³H-dopamine, for 20 min. The slices were washed with warm buffer and placed into 13-mm Swinex Millipore filter holders (1 slice/holder) for superfusion by a 4-channel peristaltic pump. The perfusion rate was 0.5 ml min⁻¹ and 2-ml fractions were collected directly into scintillation vials for counting the tritium. Slices were superfused for 20 min and then superfused with β -endorphin for 20 min before switching to KRB containing 53 mM KCl + β -endorphin (indicated by the horizontal bar), the extra KCl having replaced some of the NaCl in the regular KRB. During the collection of fraction 15, the perfusing solution was switched back to regular KRB. Tritium overflow is expressed as a percentage of the mean of 4 experiments. Preliminary studies with alumina absorption chromatography indicated that approximately 82% of the tritium in regular KRB perfusates was dopamine and this value increased to about 93% in 53 mM KCl KRB perfusates.

Table 1 Opiate inhibition of striatal tritium overflow and reversal by naloxone

Drug	Concentration	Net overflow (%)
Morphine	0	17.69 ± 2.69 (13)
Morphine	1.0 × 10 ⁻⁴ M	16.14 ± 3.42 (8)
Morphine	3.0 × 10 ⁻⁶ M	5.04 ± 1.17* (10)
Morphine	1.0 × 10 ⁻⁶ M	0.41 ± 0.25* (5)
Morphine + Naloxone	3.0 × 10 ⁻⁶ M α	16.85 ± 3.02 (8)
β -Endorphin	0	20.04 ± 2.99 (4)
β -Endorphin	0.6 × 10 ⁻⁴ M	16.76 ± 3.62 (4)
β -Endorphin	1.2 × 10 ⁻⁶ M	8.06 ± 1.60* (4)
β -Endorphin	3.0 × 10 ⁻⁶ M	0.12 ± 0.12* (4)
β -Endorphin + Naloxone	3.0 × 10 ⁻⁶ M α	10.70 ± 2.72† (4)
Enkephalin	0	18.09 ± 2.44 (7)
Enkephalin	1.0 × 10 ⁻⁶ M	20.99 ± 4.70 (8)
Enkephalin	1.0 × 10 ⁻⁴ M	17.23 ± 4.11 (5)

*Significantly different from control ($P < 0.02$)

†Significantly different from 3 × 10⁻⁶ M β -endorphin alone ($P < 0.01$).

Rat brain striatal slices were used as described in the legend to Fig. 1. Net overflow was calculated from tritium in fractions 11-15 corrected for the estimated spontaneous tritium overflow and is expressed as a percentage (mean ± s.e.) of the total tritium in the striatal slice at the beginning of the collection of fraction 11. Numbers in parentheses indicate the number of experiments.

potassium-stimulated overflow of tritium from superfused rat brain striatal slices preloaded with ³H-dopamine. Recent studies from our laboratory have shown that potassium-induced ³H-dopamine release from superfused striatal tissue from several strains of mouse which exhibit running activity in response to morphine can be inhibited by 10⁻⁴ M morphine^{11,12}. This inhibition of release seems to be a specific opiate effect because it is antagonised by naloxone, and striatal tissue from mice made tolerant to morphine by the implantation of a morphine pellet for 3 d exhibits tolerance to the inhibitory effect of morphine on dopamine release *in vitro*¹³.

Figure 1 shows the concentration-dependent inhibition of the potassium-stimulated overflow of tritium by β -endorphin from superfused rat striatal slices. Virtually complete inhibition was obtained with 3.0 μ M β -endorphin. In Table 1, the effects of morphine, β -endorphin and Met⁵-enkephalin on the net tritium overflow induced by the depolarising concentration of potassium chloride are compared. β -Endorphin was about twice as potent as morphine, the IC₅₀ for β -endorphin being 1.0 μ M and the IC₅₀ for morphine being 2.2 μ M. Met⁵-enkephalin, on the other hand,

did not produce a significant blockade of the inhibition by morphine and β -endorphin, the blockade being more complete in the presence of morphine than in the presence of β -endorphin.

In the electrically-stimulated guinea pig ileum, Met⁵-enkephalin is approximately equipotent with normorphine¹ or morphine¹², whereas in the mouse vas deferens it is about 20 times as potent as normorphine¹ or morphine¹². β -endorphin and Met⁵-enkephalin are approximately equipotent in the guinea pig ileum preparation⁶. In opiate receptor binding assays with guinea pig brain membranes, Cox *et al.*⁶ have shown that synthetic β -endorphin is 2–3 times as potent and Met⁵-enkephalin one-fifth as potent as normorphine. Thus, the order of potency of β -endorphin, morphine and Met⁵-enkephalin in the inhibition of striatal dopamine release seems to follow more closely the order in the binding and analgesia assays than in the assays utilising smooth muscle preparations.

In a very recent paper, Taube *et al.*¹⁴ have described inhibition of noradrenaline release by enkephalin in rat cortex slices. However, to our knowledge, this is the first demonstration of an inhibition of dopamine release from CNS tissue by an endogenous opiate-like peptide. The actions of β -endorphin in the central nervous system are probably not limited to the inhibition of dopamine release because it is known that opiate drugs also inhibit the release of acetylcholine^{15–18} and noradrenaline¹⁹ from CNS tissue. At present, however, dopamine is the only neurotransmitter for which tolerance to the effect of morphine *in vitro* has been demonstrated with CNS tissue¹¹ and for which an inhibition of release by morphine has been correlated, at least qualitatively, with a behavioural effect of morphine, by utilising mouse strain differences^{11,12}.

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Immunochemical evidence of cholecystokinin-like peptides in brain

A NUMBER of peptides including substance P¹, somatostatin² and vasoactive intestinal peptide³, have been found both in nerve cells of the brain and gut and in gastrointestinal endocrine-like cells. The biological significance of the occurrence of the same peptides in endocrine and nerve cells is not yet clear, although Pearse has suggested that these cell types might share a common embryological (neuroectodermal) origin⁴. An antibody to the antral hormone gastrin has recently been shown to cross react with material in extracts of the central nervous system (particularly cerebral hemispheres) of a number of vertebrate species including dog and man⁵. It is known that in antral mucosa, and in blood, gastrin occurs in several different forms of which the most important are peptides of 17 (G17) and 34 (G34) amino acid residues⁷. The C-terminal heptadecapeptide of G34 is identical with G17, and G34 may well be a biosynthetic precursor of G17 (ref. 7). The C-terminal pentapeptide of the two gastrins is also identical with that of the intestinal hormone cholecystokinin (CCK)⁸, and antibodies specific for the C terminus of gastrin frequently cross react with CCK⁹. The present study was undertaken to clarify the relationships between the gastrin-like activity in brain and the characteristic forms of gastrin and CCK. We found the distribution of the brain components on Sephadex G-25 to differ from those of previously characterised forms of gastrin and CCK. The pattern of reactivity with different antisera suggests, however, that the brain factors resembled CCK-like peptides more closely than gastrin-like peptides.

Extracts were prepared from the entire brain of rats, and from the cerebral cortex of hog and dog. Small pieces of tissue were boiled in water (0.5 g ml⁻¹) for 3 min to inactivate enzymes, homogenised, centrifuged (2,000g, 20 min) and the supernatant fractionated on calibrated columns of Sephadex G-25 or G-50. Immunoreactivity in the column eluates was estimated by radioimmunoassay using a C-terminal-specific antiserum (1296) (ref. 10).

Three peaks of immunoreactive material were found in column eluates when extracts of hog brain were fractionated on Sephadex G-25 (Fig. 1); similar results were obtained with extracts of rat and dog brain. The first peak (BP I) emerged in the void volume and accounted for 10–15% of the total immunoreactivity. Standard samples of G34 and CCK also emerged in the void volume on Sephadex G-25. But BP I differed from these standards in that it also emerged in the void volume after refractionation on Sephadex G-50, whereas G34 and CCK emerged later on Sephadex G-50 (approximately 35% and 50% respectively of total elution volume). In extracts of jejunum, and in plasma, there is material with gastrin-like immunoreactivity which emerges in the void volume on Sephadex G-50, and this component has been named 'big, big gastrin' (BBG) (ref. 11). Trypsin converts BBG to a peptide resembling G17 (ref. 11). In contrast, preliminary experiments indicated that when BP I was digested with trypsin (100 μ g ml⁻¹, 10 min) there was conversion to material emerging later than G17 on Sephadex G-25, at 60–90% of elution volume. So far only relatively small amounts of this product have been available and the identity of the material is not yet clear. Nevertheless the evidence indicates that BP I does not correspond to any of the previously characterised forms of gastrin or CCK.

The main peak of immunoreactivity in the brain extracts (BP II) eluted from Sephadex G-25 in a similar position to the C-terminal octapeptide of CCK (CCK 8); this peak accounts for over 70% of the total immunoreactivity (Fig. 1). A third peak of activity (BP III) representing 10–15%

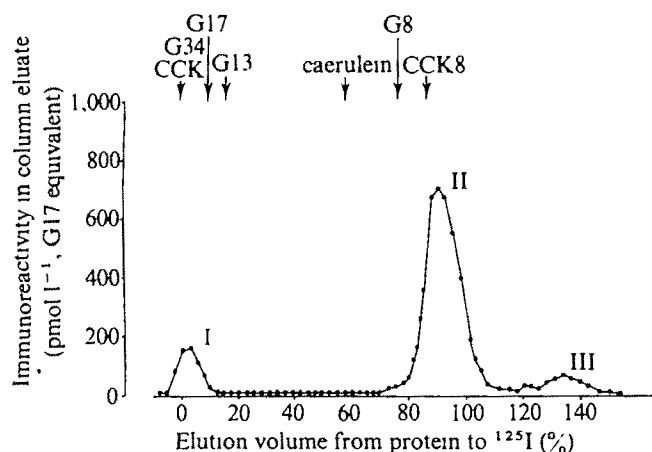


Fig. 1 Fractionation of an extract of hog brain on Sephadex G25 superfine (1×100 cm). Fractions of 1.0 ml collected every 10 min. Column eluted with 0.02 M sodium barbital pH 8.4, containing 0.02% sodium azide, at 4°C . The column was previously calibrated with 20% pure porcine CCK, pure natural human G34-I and G17-I, caerulein, synthetic peptides corresponding to the C-terminal tridecapeptide (G13) and octapeptide of G17-I (G8), and CCK 8. The elution volumes of the standards are indicated by arrows. In all column runs the void volume was marked by protein estimated by absorption at 280 nm, and the salt region was marked by Na^{125}I . Elution volume is expressed as percentage from void (0%) to ^{125}I (100%). Concentration of immunoreactive material was estimated with antiserum 1296 and expressed relative to standard G17. Peaks of immunoreactivity are identified by Roman numerals.

of the total immunoreactivity emerged considerably later than Na^{125}I (Fig. 1). Synthetic peptides corresponding to the C-terminal hexapeptide and tetrapeptide of G17 were found to emerge in the same region as BP III, but the standards were poorly resolved and it has not been possible to relate precisely their mobility to that of BP III.

The immunochemical properties of the components in brain were studied by separately pooling fractions corresponding to the three peaks and estimating concentrations of immunoreactive material by radioimmunoassay using six different antisera. The specificities of the antisera had been established by studies of the potency of standard molecular forms and synthetic fragments of gastrin and CCK in inhibiting binding of ^{125}I -labelled G17 to antiserum. Only antisera specific for the C terminus of gastrin and CCK cross reacted with the brain components. Antiserum L6, which has specificity for intact G17 (ref. 12), and antiserum 1295, which is specific for the N terminus of G17 (ref. 10), failed to cross react with extracts of the brain of rat, dog or hog, either before or after fractionation by gel filtration (Table 1). Neither 1295 nor L6 cross reacted with rat antral

gastrin so their inability to detect immunoreactive material in rat brain is not surprising. Both antisera did, however, cross react with dog and hog G17 and so the failure to detect activity in brain extracts from these species indicates that G17 could be present only in minute quantities, if at all, in the extracts. These results are therefore consistent with the failure to find a peak of immunoreactive material eluting in the same region as standard G17 when brain extracts were fractionated on Sephadex G25 (Fig. 1).

When G17 was used as a standard in the radioimmunoassays there were up to 100-fold differences in concentrations of brain components estimated with different antisera (Table 1). For each of the components the highest concentrations were recorded with antiserum C. This antiserum was raised against CCK 8 and shows twofold higher immunoreactivity with CCK 8 than with G17. On the other hand the lowest values were recorded with antiserum L2, which was raised against porcine G17 and shows 50–100 times higher immunoreactivity with G17 than with CCK 8. Two other antisera (1296, 2716) gave intermediate estimates of the concentration of brain components; both of these antisera were raised against human G17 and show 5–15 times higher immunoreactivity with G17 than with CCK 8.

Because the pattern of immunoreactivity of the brain extracts seemed to follow the degree of specificity of the antisera for CCK, the results have also been expressed relative to a CCK standard. Pure CCK was not available and so CCK 8, which contains the minimal active fragment of CCK for biological activity, was used instead. Table 1 shows that there were only relatively minor differences in estimates of immunoreactive concentration of brain components made with different antisera when based on the CCK standard. The differences between antisera were more pronounced for BP I (two- to six-fold) than for BP II and III (one- to three-fold). Taken together the results indicate that the brain components have a pattern of immunoreactivity resembling CCK-like peptides more closely than gastrin-like peptides.

An attempt to isolate these brain peptides has been initiated in this laboratory; until this is achieved absolute estimates of their concentration in brain cannot be made. Expressed in terms of CCK 8 equivalent, however, the main component (BP II) was present in amounts of $50\text{--}150\text{ pmol g}^{-1}$ in the brain of the three species (Table 2). It is unlikely that concentrations such as these could be produced other than by synthesis within the CNS. Many peptide hormones are synthesised in the form of large precursor molecules which are converted to smaller biologically active forms by proteolytic enzymes. The present results could therefore be accounted for if the three components were the product of a single biosynthetic pathway and interconversion took place by proteolysis. It is clear that the cellular origins of these components must now be studied. In addition, attention must be given to the biological activity of

Table 1 Concentration of three fractions of hog brain estimated by radioimmunoassay with six antisera

Fraction	Standard	Antiserum				1295	L6
		C	2716	1296	L2 Concentration (pmol l^{-1})		
Hog BP I	G17	156	34	34	8	<2	<2
	CCK 8	78	100	212	472	—	—
Hog BP II	G17	2,341	177	119	23	4	2
	CCK 8	1,170	520	735	1,355	—	—
Hog BP III	G17	130	14	13	1	<2	<2
	CCK 8	65	41	81	58	—	—

Each figure is the mean of assays on three separate samples of brain extracts. Samples were pooled from eluates of Sephadex G25 columns. Results are expressed relative to standard pure natural human G17-I and synthetic C-terminal octapeptide of CCK (CCK 8). ^{125}I -labelled human G17 was used as label in all radioimmunoassays. With all four C-terminal specific antisera (C, 2716, 1296, L2) dilution curves for the three brain components were parallel to those for G17 and CCK 8. Antisera 1295 and L6 do not cross react significantly with CCK 8 and so for these antisera results have been expressed relative to standard G17 only.

Table 2 Concentration of immunoreactive components in extracts of the cortex (hog and dog) or whole brain (rat)

Species	n	Concentration of components in brain (pmol g ⁻¹ : CCK 8 equivalent)		
		BP I	BP II	BP III
Hog	5	16.9±3.1	95.0±33.0	11.2±1.1
Dog	3	14.4±3.7	58.1±20.6	18.1±7.5
Rat	5	30.6±6.9	149.0±20.6	27.5±6.2

Radioimmunoassays were performed using antiserum 1296 and results expressed relative to standard CCK 8. Concentrations were estimated by integration of peaks in Sephadex G25 eluates and calculated on the basis of wet weight of tissue from which the extract was prepared.

the components and, by analogy with other CNS peptides (substance P, somatostatin), a possible role as neurotransmitters or releasing factors deserves consideration.

Technical assistance was provided by Mrs Elaine Clark, Miss Carol Higgins and G. Laing. Antisera C and 2716 were gifts from Drs S. Bloom and J. Rehfeld, respectively. Standard peptides were gifts from Professor R. A. Gregory and Dr H. J. Tracy (G34 and G17), Professor G. W. Kenner (C-terminal octapeptide, hexapeptide and tetrapeptides of G17), Dr V. Mutt (20% porcine CCK), Squibb Institute of Medical Research (CCK 8), and Farmitalia Ltd (caerulein). This work was supported by a grant from the MRC.

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Visual contrast sensitivity of a 6-month-old infant measured by the evoked potential

THE visual contrast sensitivity of the human infant has previously been measured using behavioural methods, in which psychophysical thresholds have been inferred from visual preferences¹. In this paper we report a preliminary investigation of contrast sensitivity, as measured by the amplitude of the evoked cortical response, in a 6-month-old infant. These results are compared with behavioural measurements on the same infant, and with adult data obtained psychophysically and from evoked cortical responses.

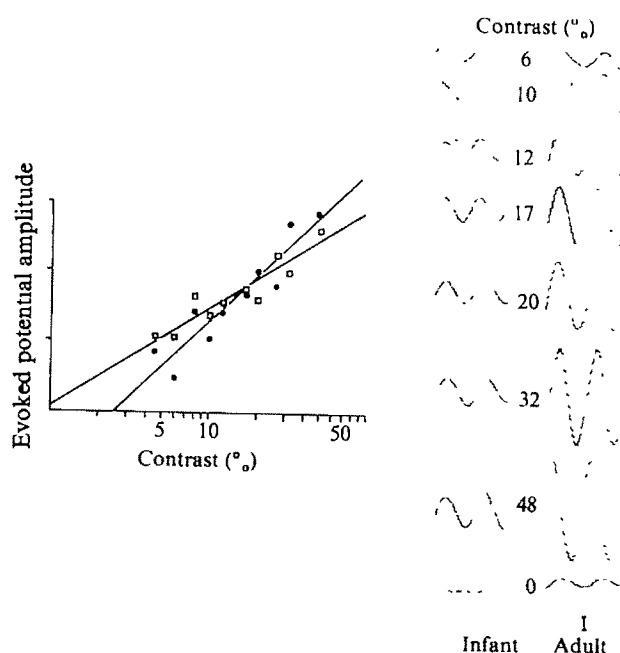
Campbell and Maffei² measured the visual evoked cortical response to a phase reversing sinusoidal grating and found that a linear relationship existed between the logarithm of contrast and the amplitude of the evoked potential. By extrapolating a regression line through such a plot to zero voltage, the psychophysical threshold could be predicted³. This relationship found in adult evoked potential measurements has been used in the present study to indicate thresholds for contrast in a situation where direct psycho-

physical methods are not possible, that is, in human infants.

The stimuli were vertical gratings of sinusoidal luminance profile, whose phase was reversed at 10 Hz. They were generated on an oscilloscope screen by established methods^{4,5}, with a mean luminance of 300 cd m⁻², and subtending an angle of 20° at 57 cm. To ensure that evoked potentials for different contrast levels were comparable in spite of possible artefactual changes over time, the various contrasts for a given spatial frequency were presented repeatedly in short blocks randomly interleaved within a particular run, which lasted about 10 min. Each block lasted 5.1 s. Control blocks, in which the contrast was set to zero, were included in each run. The infant was held sitting 57 cm from the display screen (110 cm for runs with spatial frequency higher than 5 cycles per degree). Between the infant and the display there was a 70% reflecting mirror arranged to superimpose the image of an active face on the stimuli, which served to maintain the infant's attention.

Evoked potentials were recorded from both the infant and the adult by means of 2-mm Beckmann electrodes. One electrode was placed 1 cm above theinion and the other 3 cm temporally: the signals from these were differentially amplified 100,000 times, with an earth electrode placed behind the ear. The amplified signal was filtered (by a Kemo VBF/3/J filter) with a passband of ±2 Hz centred on the second harmonic of the frequency of phase alternation (20 Hz). The filtered signal was fed into an analog input channel of a PDP-11/10 computer which maintained separate averages of the signal for each contrast level used, and also controlled the stimulus contrast and phase alternation. These averages were of a 100-ms sweep and therefore contained two cycles of the second harmonic signal. The first 100 ms of each 5.1-s block were not included in the average, to avoid the effect of initial transients due to the contrast change. The averaging could be interrupted when the infant was not attending to the screen.

Fig. 1 Right, averaged evoked potentials (500 sweeps) for a series of contrast levels, from the infant and adult subjects. Spatial frequency: 0.3 cycles per degree. The bottom traces were obtained with no grating present. Scale: 200 × 10⁻⁹ V per sweep (adult), 50 × 10⁻⁹ V per sweep (infant). Left, plot of amplitude of these potentials against contrast (logarithmic scale). ●, Adult data; □, infant data. Regression lines are shown for each subject. The amplitudes have normalised for this plot.



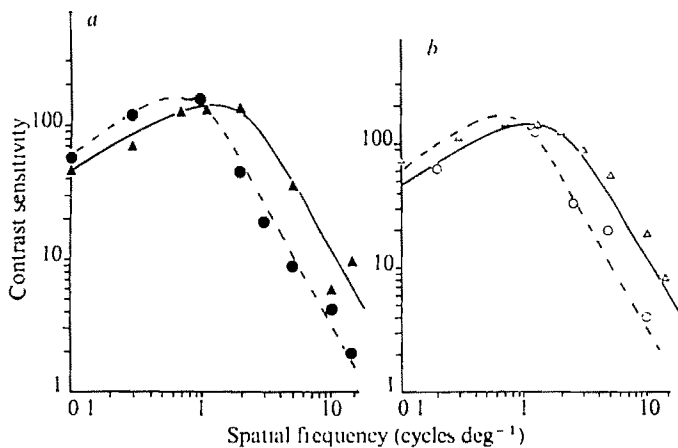


Fig. 2 *a*, Comparison of the contrast sensitivity functions for the adult (Δ) and infant (\bullet), as derived from the amplitude of the evoked potential. The curves are fitted by eye. Each point is an average of several runs. *b*, Comparison of the psychophysical contrast sensitivity for the adult (Δ) and the behaviourally determined contrast sensitivity function for the infant (\circ). The curves from Fig. 2 *a* are repeated in this figure to aid comparison between psychophysical and evoked potential measures.

In general 500 sweeps were averaged at a given contrast, although for some low contrasts extra blocks were included in the program to obtain a signal of sufficient amplitude. The averaged signals, which because of the narrow pass-band were approximately sinusoidal, were displayed, plotted on an X-Y plotter, and the peak-to-trough amplitude was put out by the computer. Signals have only been included in the results if their peak-to-trough amplitude was at least 20% higher than that measured in the average for the zero contrast control blocks.

The subjects were tested for responses to spatial frequencies ranging from 0.1 to 15 cycles per degree, and contrasts ranging from 4% to 50%, for each frequency. Psychophysical thresholds for the adult were measured with the same apparatus, using a yes-no forced choice method with a double random staircase procedure under the computer's control. The trials near to threshold were interleaved with high contrast displays in a similar sequence to that used for the evoked potential measurements and the screen was viewed through the 70% mirror. Behavioural data on the infant were gathered using the method of fixation preference assessed by two-alternative forced choice by a 'blind' observer*. Stimuli were presented on two oscilloscopes at a distance of 40 cm from the infant, subtending 15° each and separated by 9°. One screen displayed a horizontal test grating and the other a blank field matched in mean luminance. The behavioural threshold was defined as the contrast level at which the observer could make 70% correct judgements of the side to which the stimulus was presented. This was obtained by a modified staircase procedure.

Refraction of the infant subject showed that he was about 1 D hypermetropic with 0.75 D of astigmatism in each eye. He should therefore have had no difficulty accommodating to the stimulus distance. The adult subject was emmetropic.

Figure 1 shows a series of averaged signals for a single spatial frequency (0.3 cycles per degree) and increasing contrast for both the infant and adult subjects. The peak-to-trough amplitudes of the signals of each series were plotted against log contrast and the linear regression line extrapolated to give the contrast for zero amplitude. These extrapolated contrasts are plotted in Fig. 2 *a* as a function

of spatial frequency, for both the infant and the adult subjects. Curves have been fitted to these by eye. Plotted in Fig. 2 *b* are the psychophysically or behaviourally obtained contrast sensitivities for each subject, with the curves of the evoked potential results from Fig. 2 *a*.

For the adult, the extrapolated evoked potential "threshold" estimates lie close to, but in most cases slightly below, the psychophysical thresholds. This is compatible with previous findings^{2,3}. The behavioural measure on the infant gives a lower limit on the estimate of sensitivity. This in fact coincided rather closely with that derived from the evoked potential measurements (Fig. 2 *b*).

In the case of the low spatial-frequency stimuli, it is not possible to determine from our data whether the evoked potentials are generated by the spatial pattern, or by a frequency-doubled response to flicker⁷. When modulation is both spatial and temporal, however, psychophysical thresholds can also be distinguished by the detection of either pattern or flicker^{8,9}. The uncertainty about the origin of the evoked potential does not therefore exclude a comparison with the behavioural data, but it does indicate a need for caution in extending the results to different temporal conditions, such as a static pattern.

The use of evoked potentials allows us to compare the same measure of sensitivity, employed on both infant and adult. Figure 2 *a* shows that for low spatial frequencies the contrast sensitivity derived in this way is very similar for both subjects. At spatial frequencies of 2 cycles per degree and above, however, the adult is shown to be markedly more sensitive. In making these comparisons, it should be borne in mind that the infant and adult visual systems may show differences in temporal as well as spatial response.

We therefore conclude: (1) that the neural mechanisms determining contrast sensitivity at low to medium spatial frequencies have matured by 6 months of age to close to the adult level (this is consistent with the finding¹⁰⁻¹² that the optimum check size for checkerboard-evoked potentials is similar in the 6-month-old and the adult). However, the neural mechanisms determining high spatial-frequency sensitivity are not fully mature at this age. (2) Measurements of contrast sensitivity by means of the visual evoked potential are possible in infants and give data consistent with behavioural methods. It may prove a valuable clinical tool for the early diagnosis of visual problems.

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The 'cerebellum' and the control of eye movements in cephalopods

THE cybernetic principles involved in the control of locomotion in vertebrates include feed-forward and feedback between the optic and vestibular systems, through a series of control centres, including the cerebellum. Cephalopod molluscs also show swift and well controlled locomotion and have eyes and static organs, but there is little detailed evidence as to how the information from these controls movement. The basal lobes of the supraoesophageal part of the brain are known to be higher motor centres, but this is, at best, a vague term. The new studies of these lobes and of the statocyst by my colleagues and I that are reported here show more clearly the part they play in the control of behaviour. Their organisation proves to be basically similar to that of the comparable systems of vertebrates.

To the many parallels between the organisation of cephalopods and vertebrates described by Packard¹ we can therefore now add that the control of movement of the eyes and body involves a system similar to the cerebellum. The resemblances and the differences between the two systems may help towards understanding of the principles of movement control. In both there is a direct feed-forward from angular acceleration receptors to the oculomotor centre and a visual feedback system involving numerous fine parallel fibres (Fig. 1). From the verte-

Fig. 1 *a*, Diagram of the oculomotor control system in the squid arranged to show its correspondence to that of a mammal. *b*, The oculomotor control system in a mammal (copied, with permission, from Ito²). AOT, Accessory optic tract; CF, climbing fibre; CTTm, central tegmental tract; GR, granule cell; IO, inferior olive; MF, mossy fibre; PCJ, Purkinje cell; WB, unidentified brainstem nucleus; VN, vestibular nucleus

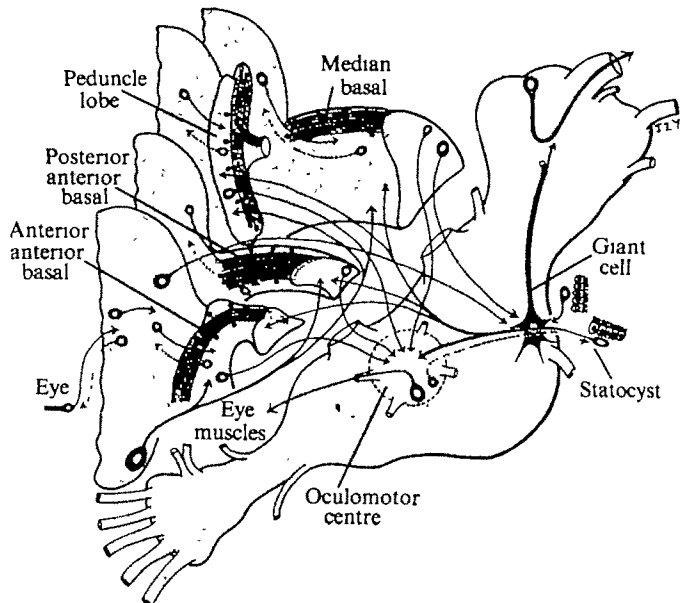
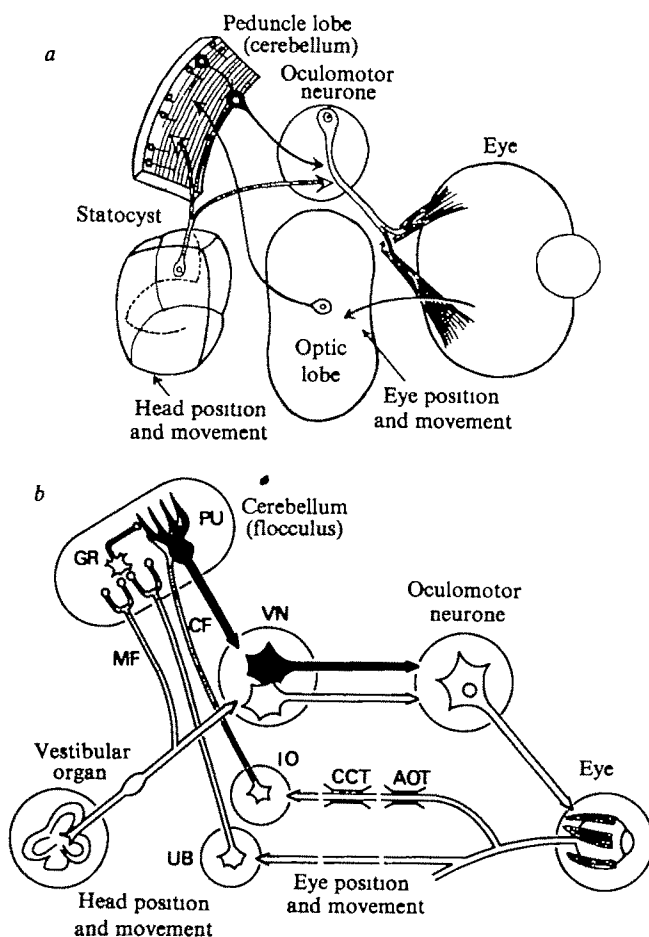


Fig. 2 Diagram of the eye-muscle control centre and basal lobe system of the squid, *Loligo*. Return centrifugal pathways are shown dotted. Note that they reach to the retinal and statocyst receptors.

brate vestibular organs, the pathway is through the vestibular nucleus²; in cephalopods fibres from the statocyst itself end in the oculomotor centre in the lateral pedal lobe (Fig. 2). It is these fibres that must be acting in the fast phase of nystagmus in *Sepia*³. In both groups the vestibular information reaches the eye muscles also through a second route in which it is combined with visual information. In vertebrates this route is through the flocculus of the cerebellum, whose Purkinje axons have been shown to exert an inhibitory influence on the neurones of the vestibular nucleus in rabbits⁴. In cephalopods the corresponding pathway is from the statocyst to each member of a set of basal lobes and from there direct to the oculomotor neurones. The visual input to the vertebrate cerebellum comes through the accessory optic tract and the inferior olive and also by mossy fibres by way of another nucleus (Fig. 1)⁵. Stimulation of the optic disk inhibits the vestibular neurones with a delay of 12 ms and of the inferior olive after 3 ms². In cephalopods the visual pathway is again through the basal lobes and there is good evidence of optokinetic nystagmus^{3,6,7}. Electrical stimulation of the basal lobes produces movements of the eyes, as well as of the head, arms and fins⁸.

Both vertebrates and cephalopods thus use a direct feed-forward control from the statocyst and a feedback from the eyes through cerebellar-type systems. The experiments of Messenger and Collewijn show clearly that the two influences together are necessary for steady visual control. They constitute a classical set of evidence for the effectiveness of such a double system.

The functional parallels between vertebrates and cephalopods make it especially interesting that the cephalopod basal lobes resemble the cerebellum in structure. There has been evidence for some time that the parallel fibres in the peduncle lobe, situated on the optic tract of cephalopods (shown in Fig. 3) resemble the granule cells of the cerebellum⁹⁻¹¹. It has now become clear that this lobe is part of a much more extensive system including the basal lobes of the brain, serving to regulate movement of the eyes and of the whole animal. The arrangement is similar in octopuses and squids but the plan has become much clearer by a recent study of squids, where some of the fibres are larger¹².

The system consists of four lobes on each side of the animal, each with a different orientation (Figs 2 and 4). The outstanding feature of each lobe is a set of parallel fibres. The region known as the anterior basal lobe contains two of these sets of

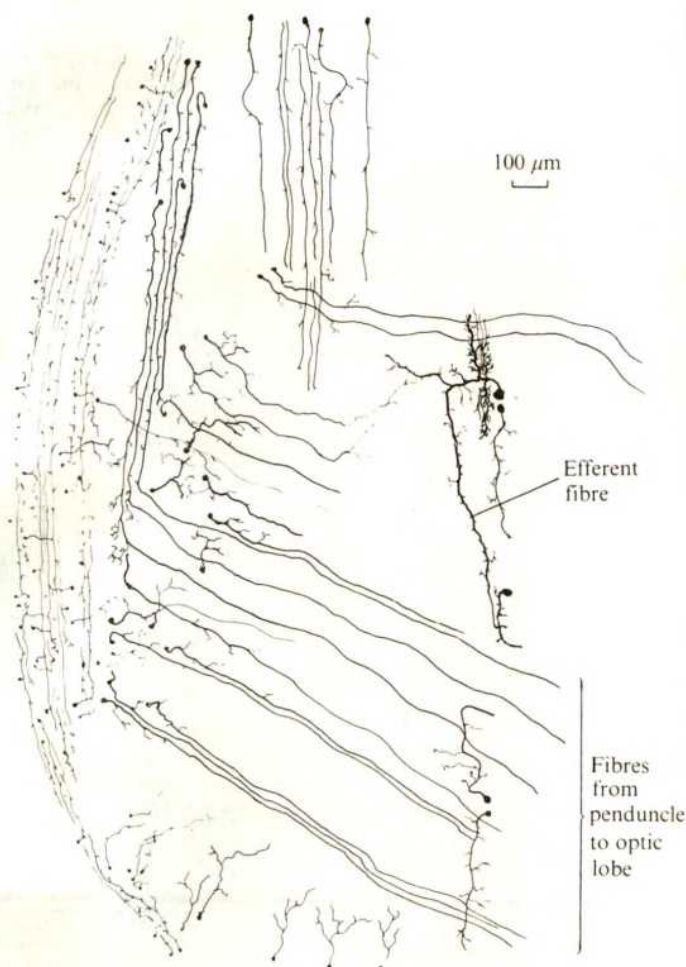


Fig. 3 Parallel fibres of the peduncle lobe of the squid (*Loligo*). Drawing from a Golgi preparation¹⁰. The optic lobe lies to the right and fibres are shown running to it. The large cell carries an output fibre with axon proceeding to the oculomotor or other motor centre. The input fibres from the optic lobe and statocyst are not shown.

fibres lying in the transverse plane. One set, the posterior anterior basal, runs transversely. The other, the anterior anterior basal, runs across the midline and then down vertically at the sides. A third set is in the median basal lobe, which although it is a more complicated region has similar parallel fibres running transversely, approximately in the horizontal plane. The fourth set of parallel fibres is in the peduncle lobe, running dorsoventrally in the sagittal plane in squids but antero-posteriorly in octopods. The four lobes together thus make a sort of 'cerebellum' with motor control functions.

These lobes all receive fibres from the optic lobes and from the statocysts (Fig. 2). They send fibres to the motor centres for the movements of the eyes and body, and others back to the optic lobes. They thus have essentially the same functional relations as the cerebellum, lying between vestibular and optic centres and serving for integrated visuostatic control of movement. It is therefore very striking to find that there are also similarities of internal organisation¹¹. The inputs of each lobe of the system in cephalopods may be listed thus. (1) Dorsal optic fibres apparently preserving the somatotopic plan of the optic lobe; it is suggested these may serve for tracking. (2) A ventral optic input of a few very large fibres from a region at the base of the optic lobe, perhaps involved with initiating attack. (3) Fibres from the statocyst. (4) Fibres from the giant cell lobe, perhaps related to escape movements. (5) Numerous fibres from the corresponding opposite lobe. (6) Numerous fibres from each of the other basal lobes.

The output is not quite the same for all the lobes but each sends to the following destinations. (1) To the oculomotor centre controlling the four eye-muscle nerves. (2) To the regions con-

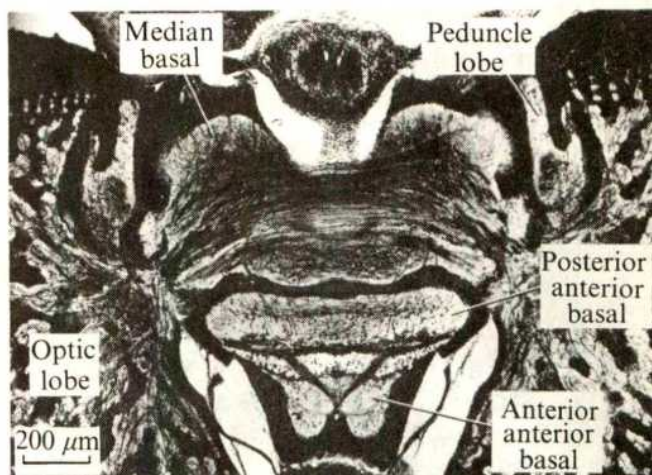
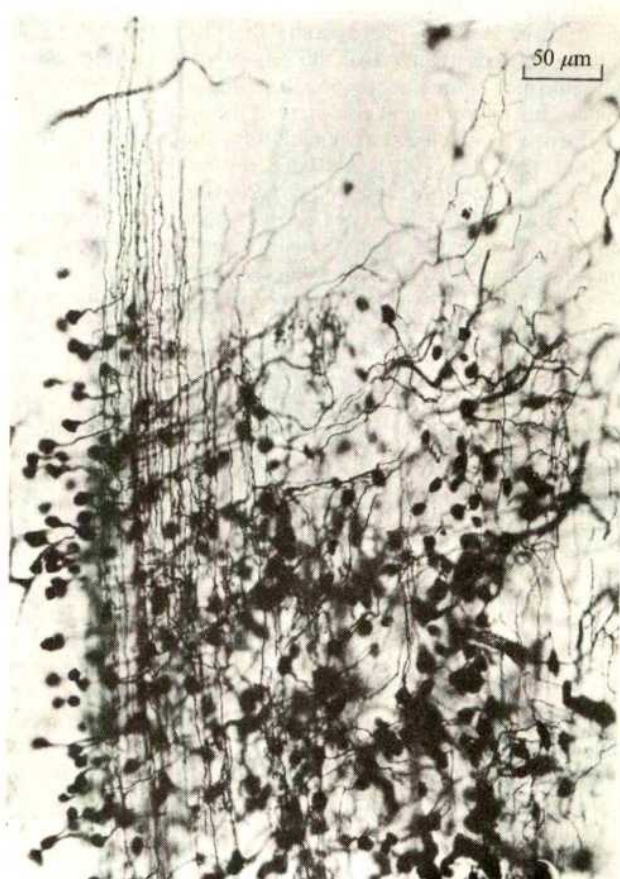


Fig. 4 Horizontal section of the supraoesophageal lobes of a small *Loligo*. Cajal stain, showing the positions of the four basal lobes on each side.

trolling the funnel and fins (for steering). (4) To the giant cells that initiate the jet. (5) A reverse projection to the optic lobe, allowing for 'efference copy' ('corollary discharge')¹³⁻¹⁵.

The details of the organisation within the peduncle lobes are beginning to be known^{9,10,16}. Some of the parallel fibres are the T-shaped processes of small "granule" cells and may run for the width of the lobe (1-2 mm) (Figs 5 and 6). They are about

Fig. 5 Golgi stained preparation of peduncle lobes of the small squid *Alloteuthis*, showing the fine parallel fibres and others crossing them.



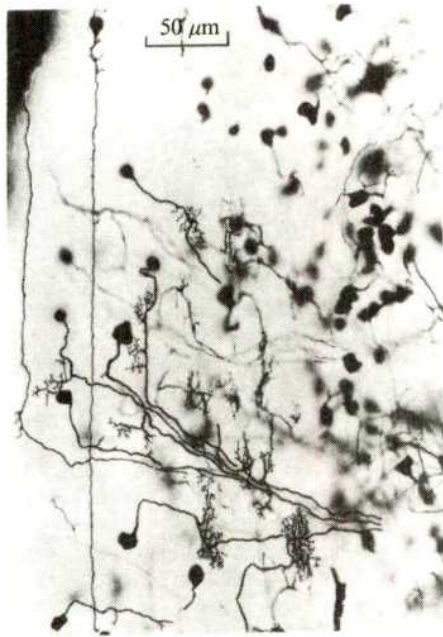


Fig. 6 Golgi preparation of *Alloteuthis*. Retouched photograph showing especially the collateral dendrites of the fibres that cross the parallel-fibre bundles.

0.3 μm in diameter and carry a series of collaterals, some making synapse with other parallel fibres. The afferents from the optic lobes branch among the bundles. Other cells have fibres that turn down across the parallel fibres to the basal region, from which the output fibres of the lobe arise (Fig. 6). These may thus serve to receive signals from the parallel fibres and pass them to the output cells, but the details of the connectivity are not yet clear. The parallel fibres in the anterior basal lobes are so similar to those in the peduncle lobes in transmission electron micrographs that it is difficult to distinguish between them¹⁶. All the sets of parallel fibres have a characteristic fluorescence, possibly due to 5-hydroxytryptamine, which is not found elsewhere in the brain¹⁷.

Evidence from electrical stimulation and surgical removal confirms that these lobes are concerned with the control of movement of the eyes and body^{9,18,19}. After unilateral lesions in the anterior basal lobe, persistent circling with the lesioned side at the centre has been seen in *Sepia*¹⁸ and *Octopus* (personal observations). After the bilateral removal of the peduncle lobes in *Octopus* there are various motor defects and movements are jerky and less precise^{9,19}.

The pattern of control of movement of the eyes and of the whole animal thus shows remarkable similarities between cephalopods and vertebrates, in spite of the fact that the gross anatomical entities are totally different. The need to monitor the animal's own movements is presumably met by the signals from the cristae of the statocyst, which run in distinct planes, providing information about angular accelerations^{20,21}. The presence of the extensive reverse pathways from the centres in the basal lobes to the optic lobes meets the need to compare performance with internally generated goal specifications. It has been common since the work of von Holst to regard this as a (relatively simple) process of subtraction. As pointed out by MacKay¹⁵, however, the process may be much more complex than this and it is interesting that the information is sent to the optic lobes, which probably act as what he would call a sensory evaluator.

The presence of systems of parallel fibres is especially intriguing and it is tempting to relate it to the hypothesis of Braitenberg²² that such systems provide for the timing of ballistically controlled movements, which are of course a marked feature of cephalopod behaviour. Whatever may be

their function the fine parallel fibres seem to be an essential feature of such systems.

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Effect of riboflavin on red-cell metabolism of vitamin B₆

THE red cell is an active site for the conversion of pyridoxine to physiological forms of vitamin B₆. Pyridoxine phosphate is first formed by a kinase and then converted to pyridoxal phosphate by an oxidase, followed by dephosphorylation to pyridoxal, the form which is released into plasma¹. In a large proportion of the patients with homozygous or heterozygous β -thalassaemia whom we have studied, there was a much reduced *in vitro* rate of red-cell conversion of pyridoxine, a familial characteristic inherited separately from thalassaemia, and also found in some haematologically normal subjects^{2–4}. This rate of conversion, whether reduced or normal, is increased by incubation of blood with riboflavin¹, possibly through synthesis in the red cell of flavin mononucleotide (FMN) from riboflavin⁵, which then stimulates the pyridoxine phosphate oxidase⁶, a flavoprotein⁷. In order to study the *in vivo* effect on red-cell metabolism of B₆, riboflavin was administered to one of the haematologically normal subjects with a greatly reduced red-cell conversion rate of pyridoxine. The rate increased to normal within 3 weeks. Here we report the details of this study.

The *in vitro* red-cell conversion of pyridoxine was studied by incubating 500 ng pyridoxine (as pyridoxine-HCl; Sigma) per ml of blood at 37 °C for periods up to 2 h as described previously¹. The *L. casei* microbiological assay was used to measure pyridoxal and pyridoxal phosphate^{1,8}. Pyridoxal phosphate cannot be measured as such, for the *L. casei* measures only pyridoxal directly. Therefore, blood was first acid-hydrolysed in order to free pyridoxal phosphate from protein and to convert it to pyridoxal. The sum of pyridoxal phosphate plus pyridoxal was therefore measured in a hydrolysate and represents the total conversion to active forms. This was expressed as a percentage of the added pyridoxine.

The subject studied was a healthy woman aged 65 yr with a haemoglobin concentration of 1.4 g dl^{-1} and with no evidence of thalassaemia. Her red-cell conversion rate was the slowest of five "slow converters" found among the control subjects. On two occasions with an interval of a year the amount converted was 1.9% and 2.3% per g $\text{Hb} \times 10^{-2}$ at 1 h which was similar to the lowest values found associated with thalassaemia^{3,4}. Her son and daughter had values intermediate between those of their mother and their father whose conversion of pyridoxine was normal. In *in vitro* studies riboflavin or FMN was incubated with blood of the subject and her son, and both compounds significantly stimulated the conversion of pyridoxine.

The *in vitro* red-cell conversion of pyridoxine in the subject was measured immediately before she started a course of riboflavin (Fig. 1). She took 10-mg tablets twice a day for 8 weeks. The conversion of pyridoxine to active forms was measured 1, 3 and 8 weeks after starting this treatment. She then stopped taking riboflavin and the conversion was measured at intervals over 17 weeks. The amount of pyridoxine converted to active B_6 forms after incubation for periods up to 2 h is compared for eight of 14 tests done during the 25 weeks (Fig. 1), and these are compared with the mean amount converted in 60 control subjects.

After one week on riboflavin the rate of conversion of pyridoxine had markedly increased. The amount converted by 1 h increased from 33 to 53% and after 3 weeks had increased further to 87%, which was equivalent to 6.2% per g $\text{Hb} \times 10^{-2}$ and was well within the normal range (5.0 to 8.6 mean 6.6% per g $\text{Hb} \times 10^{-2}$). After 8 weeks on riboflavin the conversion of pyridoxine in this subject was above the mean found in control subjects. After stopping riboflavin, the rate decreased considerably within the first 3 weeks, and continued to decrease steadily. Only by the seventeenth week, however, had the amount of pyridoxine converted at 1 h (2.3% per g $\text{Hb} \times 10^{-2}$) dropped as low as before treatment. It is interesting that the duration of her increased conversion rate corresponds approximately to the normal lifespan of red cells.

The fact that riboflavin administration resulted in a marked increase in the red-cell conversion rate of pyridoxine in this subject strongly suggests that pyridoxine phosphate oxidase is the defective enzyme. A similar though probably more rapid pattern of activation of another red-cell flavin enzyme, glutathione reductase, has been shown after ribo-

flavin administration either when the enzyme activity was originally normal or was reduced⁹. But the prosthetic group for glutathione reductase is flavin adenine dinucleotide (FAD) whereas for pyridoxine phosphate oxidase (an enzyme equally effective in the oxidation of pyridoxamine phosphate¹⁰) it was found to be FMN¹¹. The prompt response of both these enzymes to riboflavin administration is presumably explained by the fact that mature red cells take up riboflavin and synthesise the riboflavin nucleotides, FAD and FMN⁵.

The significance of the ability of the red cells to convert pyridoxine to physiological forms of vitamin B_6 is not yet understood. The considerable uptake of pyridoxine by red cells, far in advance of the rate of conversion^{1,4} suggests that a significant proportion of ingested pyridoxine is metabolised by the red cell (6% of a 40-mg oral dose as in the red cells at 20 min as well as another 3% which has passed into the plasma most probably after conversion in red cells). Vitamin B_6 is known to take part in the transport of amino acids in and out of cells^{12,13} and the red cell plays an important role in the transport of amino acids¹⁴. It is therefore possible that the conversion of pyridoxine (and pyridoxamine¹) in the red cell could be linked to amino acid transport as has been discussed at length in another paper⁴.

In vitro studies in control subjects with normal conversion rates and in patients with thalassaemia with reduced rates demonstrated an increase in the red-cell conversion rate of pyridoxine after incubation of blood with riboflavin or FMN. Detailed *in vivo* studies with riboflavin are not yet completed in these subjects, but we have shown that in β -thalassaemia the rate increases to normal in both heterozygotes and homozygotes after oral administration of riboflavin, as in the subject reported here. In a previous study we found that the red-cell conversion rate of pyridoxine was usually normal in mild homozygous β -thalassaemia (thalassaemia intermedia) but apparently was reduced in the majority of patients with transfusion-dependent β -thalassaemia¹. It is conceivable that the correction of this defect by administration of riboflavin may have some beneficial effect.

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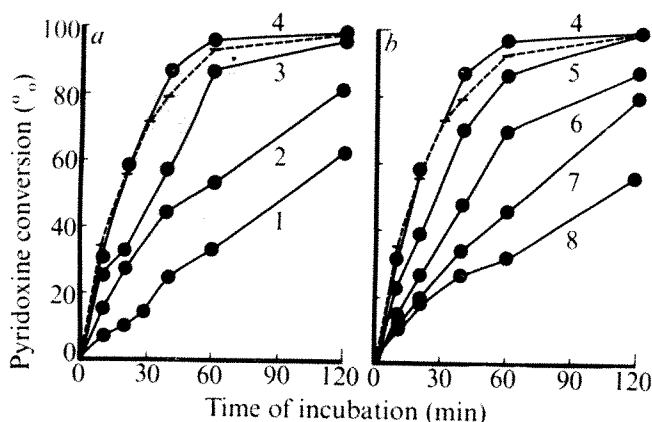


Fig. 1 *In vitro* red-cell conversion of 500 ng pyridoxine per ml blood to *L. casei* active B_6 (pyridoxal phosphate plus pyridoxal), before, during and after oral administration of riboflavin (10 mg twice daily) in a control subject with an initial slow conversion rate of pyridoxine. a, During treatment: (1) Pre, (2) 1 week, (3) 3 weeks, (4) 8 weeks. b, After treatment: (4) Pre (8 weeks treatment), (5) 1 week, (6) 3 weeks, (7) 9 weeks, (8) 17 weeks. The broken line represents the mean percentage converted by control subjects.

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Polymerisation of membrane tubulin

AMPLE evidence now exists that, in addition to soluble, cytoplasmic tubulin, many tissues contain variable proportions of particulate tubulin as judged from colchicine-binding studies¹⁻⁶. This colchicine-binding activity is not contamination from cytoplasmic microtubules, and is associated with a fraction enriched in plasma membrane markers⁶. The activity exhibits the ligand-binding properties of purified tubulin and reacts with antibodies against soluble brain tubulin. Finally, when the binding activity is solubilised from membranes it exhibits normal binding behaviour toward antimicrotubule agents, normal thermal lability, and normal interaction with antibody⁶. What was not clear was whether this tubulin could participate in polymerisation reactions to form microtubules. We report here that solubilised, labelled tubulin copolymerises with cytoplasmic tubulin through repeated cycles of polymerisation and depolymerisation.

Male guinea pigs of strain 13-N (250–300 g) were injected intraperitoneally with 5 mCi ³H-leucine (61 Ci mmol⁻¹) and whole brains were collected 60 h and 8 d thereafter for use in experiment 1 and 2, respectively. The brains were homogenised in medium containing 0.25 M sucrose, 1 mM dithiothreitol and 3 mM Tris-HCl buffer, pH 7.4. Membranes were prepared as previously described⁶ with additional washes to remove possible cytoplasmic contaminants. To check for completeness of removal of such contamination ³H-leucine-labelled cytoplasmic brain tubulin was added to unlabelled brain homogenates from which membranes were subsequently prepared. All soluble tubulin was removed after the third wash of the membranes (Table 1). In agreement with our earlier studies⁶, it seems that tubulin found in the membrane fraction is firmly attached and not an artefact of the isolation procedure.

Fig 1. Copolymerisation of solubilised membrane-bound tubulin with cytoplasmic rat brain tubulin. The ³H-labelled guinea pig brain membranes (5.2 mg ml⁻¹), isolated as described in Table 1, were suspended in 0.4% of Nonidet P-40 at 4 °C for 30 min and then centrifuged at 100,000g for 20 min at 4 °C. The supernatant, which contained solubilised membrane-bound tubulin, was mixed with an equal volume of 100,000g supernatant of rat brain homogenate and to the total mix an equal volume of 8 M glycerol in polymerisation buffer was then added. The polymerisation and depolymerisation was carried out according to Shelanski *et al.*⁷ and repeated seven times. After each cycle of polymerisation the pellet, which contained microtubules, was suspended in the minimum amount of polymerisation buffer. An aliquot was counted for radioactivity and protein was determined. Experiments 1 (curve *a*) and 2 (*b*) were carried out with guinea pig brain labelled for 60 h and 8 d, respectively.

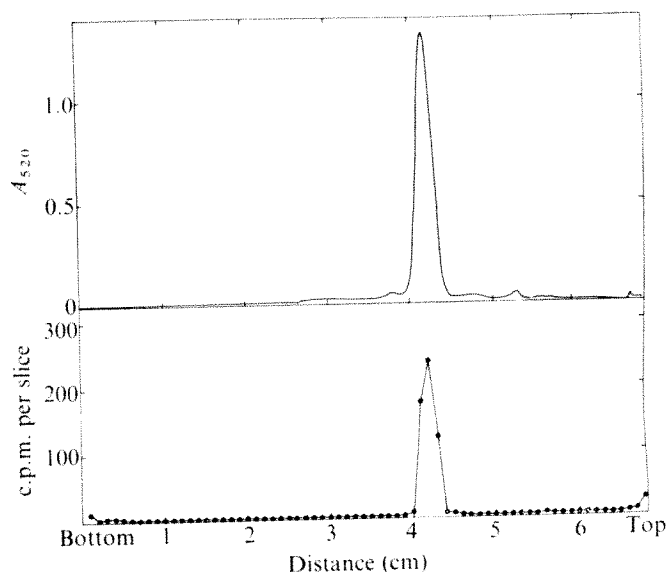
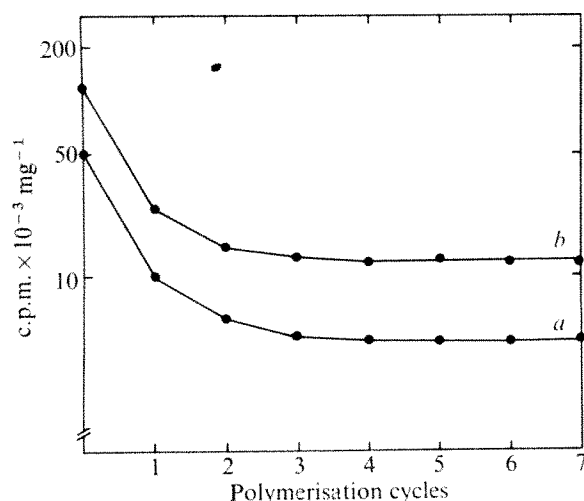


Fig. 2 SDS-disc gel electrophoretic analysis of copolymerised membrane bound tubulin with cytoplasmic rat brain tubulin. The sample analysed was from experiment 2, Fig. 1, after seven cycles of polymerisation and depolymerisation. An aliquot of 900 c.p.m. (75 µg) was electrophoresed on 5% acrylamide, 0.2% bisacrylamide and 0.1% SDS gel according to Weber and Osborne⁸ in phosphate buffer (pH 7.1). The samples were run at a current of 4 mA per tube at 23 °C until the tracking dye reached the end of the gel. The gels were stained with 0.02% Coomassie brilliant blue in 50% methanol and 9% acetic acid and destained in 5% methanol and 7.5% acetic acid. *a*, The gel was scanned at 520 nm on a Gilford spectrophotometer with a linear transport attachment; the ordinate is linear in absorbance. *b*, The gel was sliced into 1-mm disks, digested in 0.2 ml of 30% hydrogen peroxide at 37 °C for 48 h, and then counted in Aquasol.

Solubilisation of membrane tubulin using Nonidet P-40 was carried out as previously described⁶, and the soluble radioactivity was mixed with unlabelled supernatant (100,000g for 1 h) and purified through up to seven cycles of polymerisation⁷. Constant specific activity was obtained after the third cycle of polymerisation (Fig. 1). Such repolymerisation to constant specific activity is operationally equivalent to classical recrystallisation to constant specific activity and suggests strongly that the radioactivity of the membranes is either tubulin, or tau or other microtubule-associated protein.

That the radioactivity is, in fact, tubulin was verified in

Table 1 Distribution of added ³H-tubulin in different washings of crude membranes

Tissue treatment	³ H (c.p.m.)
Homogenate	12,080
Soluble supernatant	10,200
1st wash	1,100
2nd wash	200
3rd wash	25
4th wash	<5
5th wash	not detectable
6th wash	not detectable

³H-Tubulin, 12,000 c.p.m., was added to rat brain and homogenised in 3 vol membrane buffer (containing 0.25 M sucrose, 1 mM dithiothreitol, and 3 mM Tris-HCl buffer, pH 7.4). The homogenate was diluted with the same buffer to make it 10% with respect to tissue weight, centrifuged for 10 min at 700g to remove debris and the resulting supernatant solution was centrifuged at 37,000g for 10 min. The pellet was washed with membrane buffer three times and then again three times with polymerisation buffer (containing 0.1 M 2-(N-morpholine) ethane sulphonic acid (MES) (pH 6.4), 1 mM ethylene glycol bis(β-aminoethylether)-N,N'-tetraacetic acid (EGTA), 1 mM GTP, and 0.5 mM MgCl₂) before solubilisation. During the washing with polymerisation buffer, membranes were sonicated (3 s) each time to ensure complete removal of trapped cytoplasmic tubulin.

experiments where seven-cycle purified microtubules were examined by sodium dodecyl sulphate (SDS)-disc gel electrophoresis⁸. More than 90% of the protein migrated as the 55,000-dalton subunit of tubulin (Fig. 2), and all the radioactivity comigrated in this position. It seems likely, therefore, that the membrane radioactivity polymerised to constant specific activity is tubulin, and that this tubulin is normal with respect to its ability to polymerise to microtubules.

Only a small minority of electron micrographs show microtubules in close contact with plasma membranes⁹⁻¹¹, and in most cases a sizeable gap occurs between the ends of microtubules and the membrane. Similarly, freeze-fracture preparations of membranes provide no evidence for microtubules embedded in the membrane. Nevertheless, numerous models have been proposed in which microtubules directly modify membrane behaviour¹²⁻¹⁴. What then is the function of tubulin embedded in the membrane? One possibility is that it acts as a nucleation centre for microtubule polymerisation and that this region of the microtubule does not survive the usual fixation for electron microscopy. Indirect evidence that this may be so has been provided by Becker *et al.*¹⁵ who showed specific, temperature-sensitive, non-radiative energy transfer between fluorescein-labelled membranes and rhodamine-labelled tubulin. Whatever its role, the tubulin bound to membranes seems to be identical to cytoplasmic tubulin in all respects, except that it has greater thermal stability, and must be taken into account in interpreting the effects of antimicrotubule drugs on membrane behaviour.

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Operon controlling motility and chemotaxis in *E. coli*

THERE has been extensive study of the genetic basis for flagellar formation^{1,2}, motility³ and chemotaxis^{4,5} in *Escherichia coli*. But in order to understand the specific mechanisms involved in these processes it is necessary to identify the gene products. We recently showed how *E. coli*- λ hybrids could be used to identify flagella-related proteins⁶ and we have subsequently isolated two phages that carry the genes directly involved in flagellar functions. One phage (Fig. 1a, λ fla2) carries *flaI*⁷ which regulates the synthesis of flagellar proteins and *mot*,

which is necessary for flagellar rotation. λ fla2 also seems to carry part of the *cheA* gene⁸, which is concerned with regulating flagellar rotation in response to chemotactic signals. It is thought to carry only part of the *cheA* gene because the phage does not show genetic complementation when it is introduced into *cheA* mutant strains. It does, however, produce wild-type recombinants with one *cheA* mutant (e14t1) but not with another (e14q1) (Fig. 1b). Furthermore, the hybrid- λ phage that carries the adjacent segment of DNA (Fig. 1a, λ fla3) also does not show complementation when it is introduced into *cheA* mutant strains. It gives wild-type recombinants with the *cheA* mutant e14q1 but not with the strain that carries the allele e14t1 (Fig. 1b). On the basis of these observations we concluded that the endonuclease (*EcoRI*) which was used to generate the DNA fragments that were cloned cleaved the *cheA* gene, and that λ fla2 carries the promoter proximal part of the gene⁸ while λ fla3 carries the distal part of the gene (Fig. 1a).

Two polypeptides FP39 (flagellar protein, molecular weight 39,000) and FP31 were synthesised after infection of ultraviolet-irradiated cells with λ fla2 and its derivatives. FP31 was identified as a product of the *mot* gene but no assignment could be made for FP39. Two possibilities were considered⁹: (1) that it was a polypeptide fragment representing the promoter proximal part of the *cheA* gene or (2) that it was the product of a second *mot* cistron which mapped between *mot* and *cheA*. To distinguish between these possibilities, a new phage was constructed that restored the continuity of the *cheA* gene. The first hypothesis predicts that the polypeptides coded for by this phage will include a new larger polypeptide replacing FP39. The second hypothesis predicts that FP39 will remain and that an additional polypeptide corresponding to the integral *cheA* gene will appear.

The steps involved in the construction of the new phage are summarised in Fig. 1a. λ fla18 was derived from λ fla2 and λ fla18 Δ 2 carried a deletion which removed the *flaI* gene and part of the *hag* gene as well as one *EcoRI* site⁸. The *mot* gene and part of the *cheA* gene (Fig. 1b) remained fused to the right arm of lambda. λ fla42 was derived from λ fla3, a deletion removed the *flaG* and *flaH* activities. λ fla42 Δ 13 carried a further deletion which removed the *cheB* gene activity as well as an *EcoRI* site leaving the other part of the *cheA* gene fused to the left arm of lambda. *EcoRI* endonuclease digestion and subsequent ligation was used to construct a new phage (λ fla52) composed of the left arm of λ fla42 Δ 13 and the right arm of λ fla18 Δ 2. The new phage carried *mot* and could form recombinants and showed complementation with *CheA*⁻ strains (Fig. 1b). Even in liquid culture *CheA*⁻ strains that showed the characteristic smooth swimming phenotype regained the ability to 'tumble' within 25 min after infection by λ fla52. Thus we conclude that λ fla52 carries the gene that can restore functional *cheA* activity.

Figure 2 shows the flagellar proteins that are synthesised after infection with hybrid λ phages. λ fla42 specifies the synthesis of the *cheB* gene products (including FP38 and FP8), the *hag* gene product, the triplet protein(s) (FP62) and a low molecular weight polypeptide (FP12). The *cheB* gene products, the "triplet" protein(s) and the low molecular weight polypeptide will be discussed in detail elsewhere. For our present purposes the "triplet" bands serve as a convenient outside marker on the left-hand part of λ fla52. Infection with λ fla42 Δ 13 results in the "triplet" and flagellin bands while λ fla18 Δ 2 gives rise to FP31 and FP39 as well as a new band FP43, which is associated with the deletion that removes flagellin activity and serves as a convenient marker for the right arm of lambda. λ fla52 specifies the synthesis of three new bands that are flagellar specific and could not have been predicted from the properties of the parent phages. The bands will be referred to as FP76, FP66 and FP12.

The restoration of the continuity of *cheA* resulted in the appearance of new polypeptides but did not affect FP39. This suggests that FP39 is not a product of the *cheA* gene but rather results from another gene between *cheA* and *mot*. Armstrong and Adler³ showed that strains carrying *mot* mutants fell into

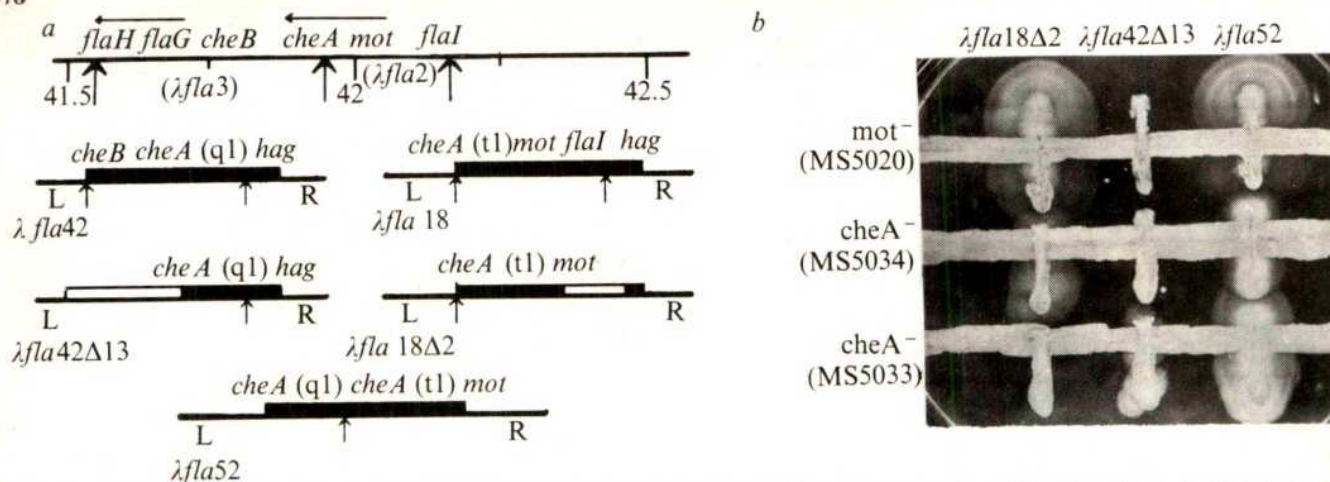


Fig. 1 a. Construction of λ-hybrid phage carrying intact Mocha operon. The top line shows a portion of the *E. coli* genetic map. Arrows below the line indicate the position of *EcoRI* endonuclease sites. The construction of λfla3, λfla2, λfla18, λfla1Δ4 as well as the methods for genetic characterisation and endonuclease mapping have been described^{6,8}. λfla42 was made from λfla3Δ1 (a deletion of λfla3 which removed *flaG* and *flaH*) and λfla1Δ4. Deletions were selected by the method of Parkinson and Huskey¹². The heavy line indicates the presence of the appropriate gene, detected by complementation or recombination. CheA(t1) and cheA(q1) refer to the ability to form recombinants with strains carrying these alleles. The light areas indicate specific deletions. λfla52 was constructed by mixing *EcoRI* endonuclease-treated DNA from λfla42Δ13 and λfla18Δ2 followed by annealing and ligation. After transfection 400 plaques were picked and tested. They had the genetic properties of all four possible combinations, λfla42Δ13, λfla18Δ2, λfla1Δ4 and λfla52. Three plaques with identical properties were isolated: λfla52, λfla55 and λfla57. **b.** Genetic characterisation of λfla52 and its parent phages. The phages were streaked across tester strains⁸ carrying a *mot* mutation (MS5020, a λ lysogen of² MS797). A *cheA* mutation (MS5034, a λ lysogen of e14t1) and another *cheA* mutation (MS5033 a λ lysogen of e14q1) (both obtained from J. S. Parkinson). Recombination is recognised by the formation of swarms (diffuse spreading and concentric circles); complementation is scored by the dense short trails close to the region of infection. For example, the infection of MS5020 by λfla18Δ2 shows both recombination and complementation, MS5034 shows only recombination and MS5033 shows neither.

two groups, *motA* and *motB*. They could not, however, exclude the interpretation that these represented intracistronic complementation within a single *mot* gene. In *Salmonella*⁹ there are apparently two cistrons, *motA* and *motB*. We tested a large number of deletions of the phage for complementation and recombination with strains carrying *motA*, *motB* and *cheA* mutations. Figure 3 shows that the deletions of λfla52 could

distinguish clearly between *motA* and *motB* mutations. Furthermore, with the exception of λfla52Δ7 (which probably represents a deletion within the *motA* structural gene) deletions into *motA* (λfla55Δ1) resulted in loss of expression of *motB* and *cheA* function, although deletions into *cheA* or *motB* had no effect on *motA*. This kind of polarity can best be interpreted in terms of an operon in which the order of transcription is

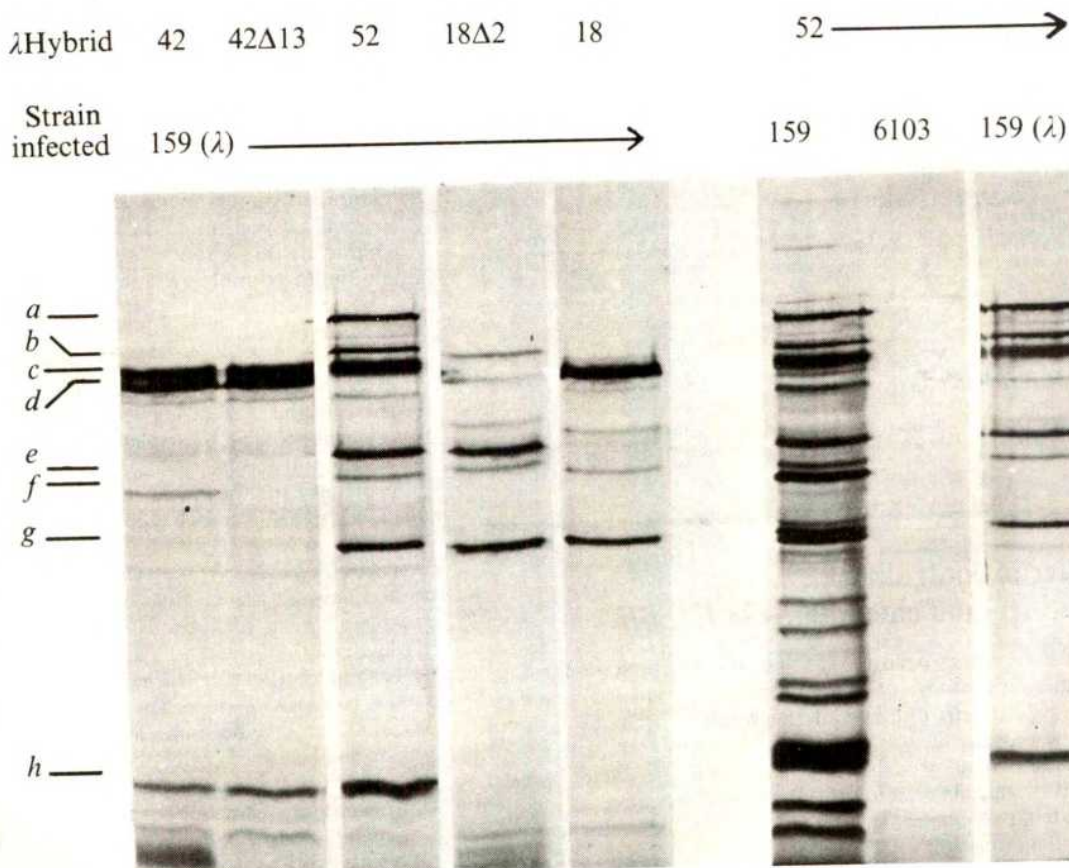


Fig. 2 Electrophoresis of proteins synthesised in ultraviolet-irradiated cells infected with hybrid λ. Conditions of infection, labelling electrophoresis and autoradiography have all been described^{6,13}. The letters on the left of the figure refer to the bands on the gel: a, FP76; b, FP66; c, triplet, FP62; d, flagellin; e, FP39; f, FP38; g, FP31; h, FP12. Infection of strain 159 with λfla52 results in the expression of proteins coded for by λ as well as those specified by λfla52. Strain 6103 carries the *flaI* mutation and is also a lysogen. The *flaI* mutation specifically represses the synthesis of flagellar gene products and the resident λ represses λ-specific gene products¹³.

motA, *motB* and *cheA*⁸. We refer to this grouping as the Mocha operon. The "triplet" product is not included in the Mocha operon.

Further evidence for an operon-like unit comes from observations on the effects of the deleted phages on the behaviour of *mu*-induced *mot* mutants. When the *mu* genome is inserted in the *motA* gene it eliminates both motility and chemotaxis (J. S. Parkinson, personal communication). When λ *fla52Δ1* (*MotA*⁺ *MotB*⁺ and *CheA*⁺) infected the *mu mot* mutants it restored both motility and chemotactic behaviour (tumbles) in less than 30 min. λ *fla57Δ5* (*motA*⁺, *motB*⁺) restored motility but the cells showed the smooth swimming phenotype characteristic of *CheA*⁻ mutant strains. Neither λ *fla52Δ7* nor λ *fla52Δ11* restored motility, thus both the *motA* and *motB* products are necessary for motility.

The polypeptides that are determined by the deleted phages are shown in Fig. 3. Band FP31 corresponds to *motA*, FP39 corresponds to *motB* and FP76, FP66 and FP12 all correspond to *cheA*. The three *cheA* polypeptides could result from processing of a single polypeptide or alternatively there may be three contiguous genes that code for these polypeptides. In order to distinguish between these possibilities heteroduplexes between λ *fla52Δ7* DNA and λ *fla52Δ1* DNA were formed. λ *fla52Δ1* deletes the triplet band which should map on the left side of the *cheA* gene and should be contiguous with the longer left arm of lambda. In λ *fla52Δ7* only the *motA* activity is deleted, which suggests a small deletion on the right side of the

Fig. 3 Correlation between genetic properties and specific protein synthesis. The table on the top summarises the results of complementation and recombination tests: +, complementation and recombination; R, recombination alone; —, neither. The strains MS5037 (a λ lysogen of strain 448 *motA*), MS5038 (a lysogen of 507 *motB*) and MS5039 (λ lysogen of 483 *motB*) (all obtained from J. Adler) were originally isolated and characterised by Armstrong and Adler³. The acrylamide gel bands are referred to in the same way as in Fig. 2. The intense band above the one labelled *e*, is FP43.

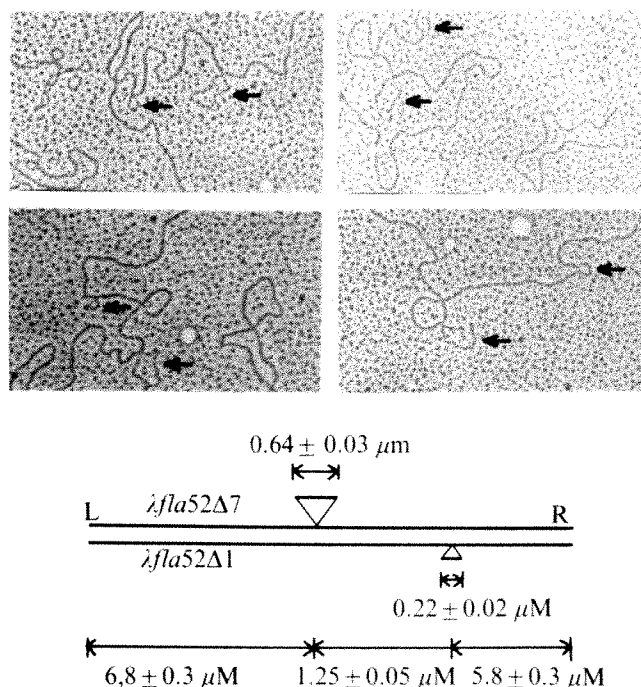
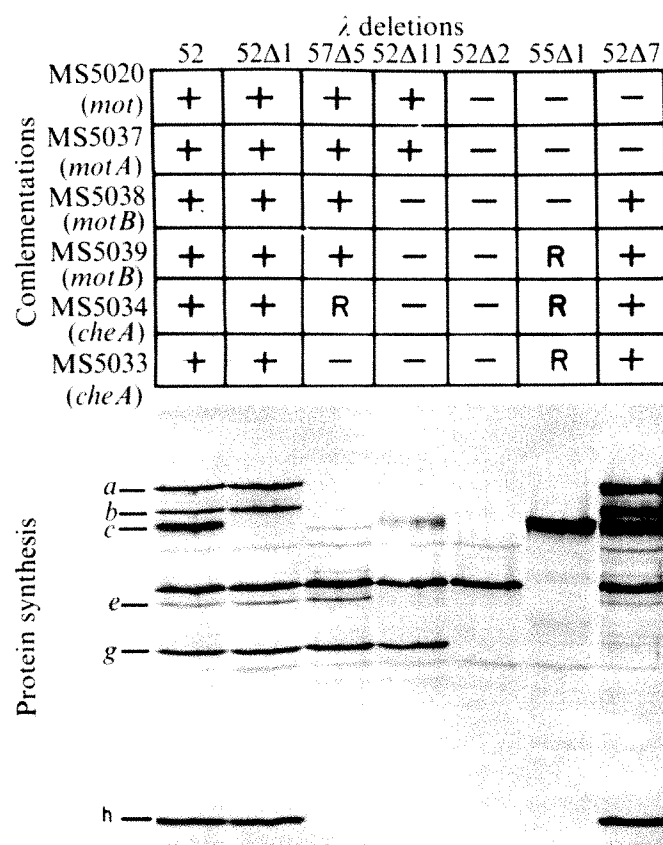


Fig. 4 Heteroduplex maps of λ *fla52Δ7* and λ *fla52Δ1* DNA. Heteroduplex mapping was done according to the procedure of Davis¹⁴: 25 molecules carrying the double loops were photographed in the presence of circular colicin E1 DNA (2.14 μ M). The photographs were traced and the relative contours calculated with a Hewlett-Packard calculator digitiser. The col E1 DNA was used as reference.

motB gene contiguous with the shorter right arm of lambda. The region between these two deletions corresponds to the *motB* and *cheA* genes. Twenty-five heteroduplex molecules with double loops were measured; the results are shown in Fig. 4. The distance between the deletion loops corresponds to 8.5% of lambda, or to 3.7 kb pairs, or to 1,230 amino acids. If all of the DNA is transcribed and translated into protein, this must account for both the *motB* and *cheA* polypeptides. Thus the *cheA* gene product could not have more than 835 amino acids. This is slightly more than half the number of amino acids required to make up three polypeptides with apparent molecular weights of 76,000, 66,000 and 12,000.

We conclude that the *cheA* gene is part of a cotranscribed unit, Mocha. The gene products resulting from this operon include proteins of molecular weight 31,000 and 39,000 that correspond to the *motA* and *motB* cistrons and three polypeptides of molecular weight 76,000, 66,000 and 12,000 that correspond to the *cheA* region of the genome. This region does not have the contiguous coding capacity sufficient to account for unique polypeptides of these sizes. There are various mechanisms that could rationalise this apparent discrepancy, for example, modification or processing of a single precursor polypeptide, or modifications in the transcription or translation processes¹⁰. Analysis of the peptide maps should distinguish between these possibilities.

In any event, if all three polypeptides have a function in chemotaxis, it may help to explain the complexity in the complementation patterns observed with *che* mutants in *E. coli*⁶ and *Salmonella*¹¹.

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Ends of bacteriophage Mu DNA

THE temperate bacteriophage Mu does not prefer a specific attachment site on the chromosome of *Escherichia coli* and can apparently engineer the insertion of its DNA irrespective of the host sequences encountered¹. The highly promiscuous, non-homologous integrative recombination between Mu DNA and *E. coli* DNA has prompted a comprehensive analysis of the structure of the Mu genome. The DNA molecules in mature Mu virus particles are linear duplexes of 37-38 kilobases (kb) (ref. 2). When the two Mu DNA strands are separated and reannealed, complete duplexes are not regenerated. Instead, one end is always split into two single-stranded tails, which can be readily seen with an electron microscope^{3,4}. This end of Mu DNA has been identified as the right end or the S end⁵. The single-stranded tails are generally 1.5 kb in length, although occasionally the single strand can be as short as 0.5 kb or as long as 3.0 kb (ref. 6). The non-renaturation of the S end clearly reflects the heterogeneity of the terminal sequences. Studies on the renaturation kinetics of the Mu DNA have implied that *E. coli* sequences at the S end are responsible for the heterogeneity⁷. Allet and Bukhari have proposed that the left end of Mu DNA, the c end, is also not fixed and varies in length by about 100 base pairs⁸. This proposal stemmed from the observation that the DNA fragment cleaved from the c end of Mu by the restriction endonuclease *HindIII* (from *Hemophilus influenzae*) does not give a sharp band on polyacrylamide or Agarose gels after electrophoresis. The heterogeneity of the c end is normally not detectable by electron microscopic techniques.

Bukhari and Taylor have presented evidence showing that the heterogeneity at the S end is a consequence of the headful packaging of Mu DNA from maturation precursors which have *E. coli* DNA covalently linked to the phage DNA⁹. The packaging reaction starts in an oriented manner from the c end and proceeds toward the S end. This inference is supported by partial denaturation mapping studies of Inman *et al.*⁹ They have demonstrated that the S end of Mu is situated at the phage head-tail junction. We report here experiments which demonstrate conclusively that *E. coli* DNA is present at the c end as well as the S end of Mu. We further show that the end sequences represent different parts of the *E. coli* chromosome and that the amount of host sequences at the c end is about tenfold less than those present at the S end.

We hybridised radioactively labelled *E. coli* DNA to the Mu DNA fragments generated by various restriction endonucleases. These experiments were based on the technique

of Southern¹⁰, in which DNA fragments from the Agarose gels are transferred to a nitrocellulose paper and then hybridised with the radioactive RNA or DNA probe. Autoradiography then reveals which, if any, fragments give significant specific hybridisation with the probe. If Mu contains *E. coli* DNA at the ends, only the two terminal fragments would hybridise with the *E. coli* DNA whereas the internal fragments would not.

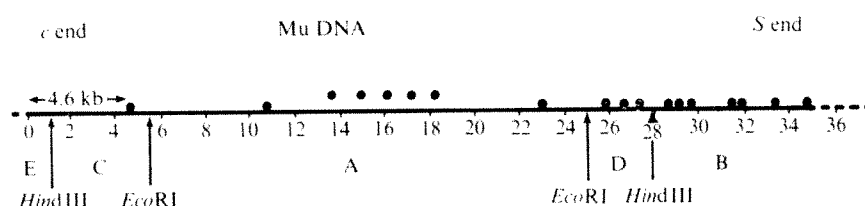
We cleaved Mu DNA with the restriction endonucleases *HindII* and *HindIII* and *EcoRI* and separated the resulting fragments by electrophoresis in Agarose gels. The sites at which these enzymes cut Mu DNA are shown in Fig. 1 (ref. 8). Both *HindIII* and *EcoRI* are known to cleave Mu DNA at two sites. *HindIII* gives a diffuse fragment of about 1.1 kb from the c end and a fragment of about 7 kb from the S end. *EcoRI* produces a fragment of about 5.1 kb from the c end and of about 10 kb from the S end. *HindII* cleaves Mu DNA at 17 sites, the c end fragment being 4.5 kb in length. No S end fragment could be seen, however, with *HindII*. Presumably *HindII* cuts too close to, and within, the heterogeneous sequences resulting in a whole range of different size fragments.

The hybridisation of ³²P-labelled *E. coli* DNA to Mu DNA fragments generated by *HindII* is shown in Fig. 2. The fragments were first stained with ethidium bromide, photographed under ultraviolet light and then transferred to a nitrocellulose paper for hybridisation. It can be seen in the autoradiograph that only one Mu fragment hybridised with the *E. coli* DNA. We know that this fragment, about 4.5 kb in length, comes from the c end² and as expected is further cleaved by *HindIII*. To show the presence of host sequences at the S end, the fragments generated by *HindIII* and by the combined action of *HindIII* and *EcoRI* were hybridised with ³²P-labelled *E. coli* DNA. As shown in Fig. 3, the fragment representing the S end as well as the fragment representing the c end specifically hybridised with *E. coli* DNA, whereas the other fragments did not.

Because of the heterogeneity of the Mu DNA ends, it is logical to assume that the host sequences do not arise from a specific segment of the host chromosome but that they are derived from all parts of the host genome. To test this assumption, *E. coli* DNA was cut with *EcoRI* or *HindIII* and the fragments were hybridised with ³²P-labelled Mu DNA. Figure 4 shows that whereas calf thymus DNA showed no hybridisation with Mu DNA, all *E. coli* DNA fragments picked up the ³²P label. The general hybridisation could be seen particularly with the larger fragments of *E. coli* DNA in the upper part of the gel. Hybridisation of *EcoRI* fragments of *E. coli* with the purified ³²P-labelled S end and the c end fragments provides results similar to the whole Mu DNA. It can be inferred therefore that all parts of the *E. coli* genome are represented at the ends of Mu DNA. Whether all parts have an equal chance of being present at the end cannot be determined precisely at this time. It can be seen in Fig. 5 that some host fragments stand out more clearly above the general hybridisation background. It is not clear whether these host segments are overrepresented at the ends or whether Mu DNA also has some specific sequences related to sequences in *E. coli*.

The relative amounts of host DNA at the c end and at

Fig. 1 Cleavage map of Mu DNA. Mu DNA is drawn as a solid line calibrated in kilobases. The broken line at the end represents host DNA. The sites at which *HindIII* cleaves Mu DNA are shown by solid circles. The circles above the line indicate *HindII* sites, in the middle of Mu DNA, which have not been ordered with respect to each other. The c end fragment produced by *HindII* is shown as 4.6 kb long segment. Cleavage sites of *HindIII* and *EcoRI* are shown by arrows. A-E, Fragments generated in the presence of both *HindIII* and *EcoRI*.



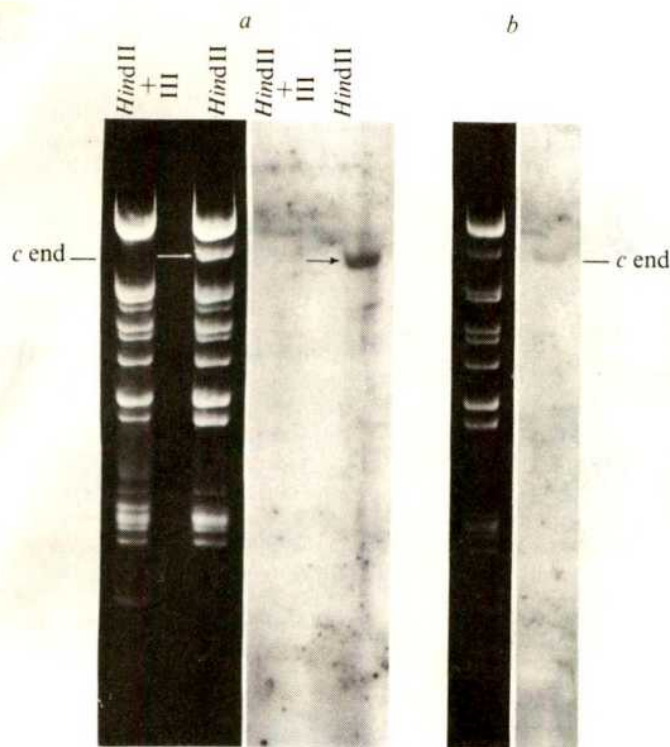


Fig. 2 Hybridisation with *E. coli* DNA to Mu DNA fragments generated by *Hind*II and *Hind*II + *Hind*III. Bacteriophage Mu particles were purified by caesium chloride density gradient centrifugation; the DNA was extracted with phenol and digested with *Hind*II and *Hind*II + *Hind*III (refs. 6 and 8). The fragments were separated by electrophoresis in 1.4% Agarose slab gels, stained with ethidium bromide and photographed under ultraviolet light, as described by Sharp *et al.*¹¹. To denature the fragments, the gels were soaked in 0.2 M NaOH, 0.6 M NaCl for 40 min and this solution was then replaced by 0.6 M NaCl and 1 M Tris, pH 7.4, for 30 min. After denaturation and neutralisation the fragments were transferred to nitrocellulose paper (Millipore 25 HAWP) as described by Southern¹⁰. The gel strip containing the denatured fragments was placed on a large sheet of Whatman MM paper, wetted with 4×SSC (SSC = 0.15 M NaCl, 0.15 M Na citrate), on a glass plate. A strip of nitrocellulose paper, a strip of Whatman MM paper and some absorbent papers, in that order, were laid on top of the gel strip and the large sheet of Whatman paper was continuously wetted with 4×SSC, with frequent changes of the absorbent papers. The DNA became trapped in the nitrocellulose paper as 4×SSC passed through the gel, drawn by the filter paper¹⁰. After the transfer of the fragments was complete (about 3 h), the nitrocellulose paper was dried, baked in an oven at 65 °C and soaked for a few hours in Denhardt's solution¹² to reduce nonspecific binding of labelled DNA. The hybridisation with denatured ³²P-labelled *E. coli* DNA was then carried out using the standard method of DNA-DNA hybridisation on filters¹². The ³²P-labelled *E. coli* DNA was prepared by growing *E. coli* K12 cells in a low phosphate medium, supplemented with H₃³²PO₄. The hybridisation mixture contained 0.1–0.5 mg ³²P-labelled denatured probe DNA (generally with 2–5 × 10⁵ c.p.m.), 0.5% SDS, in 1× Denhardt's solution and 6×SSC. After hybridisation the nitrocellulose paper was washed extensively in 2×SSC + 0.5% SDS at 60 °C, dried and autoradiographed. For further technical details see ref. 13. The figure shows Mu DNA fragments visualised after ethidium bromide staining (left hand columns) and after hybridisation with ³²P-labelled *E. coli* DNA (right hand columns) Mu *cts62(a)* was grown by induction of strain BU 165 which contains a single copy of Mu *cts62 mom*⁶. Mu *c3(b)* was grown by infection. The position of the *c* end fragment with *Hind*II is indicated by an arrow. This fragment is cut by *Hind*III and thus does not appear in the *Hind*II + *Hind*III digest of Mu *cts62* DNA. The *S* end fragment is too heterogeneous to be seen in these conditions*. Hybridisation with the *c* end fragment is visible in the autoradiograph. Hybridisation with the diffuse *c* end fragment generated by *Hind*III (labelled *Hind*IIIc) is too low to be detected here and is shown in Fig. 4.

the *S* end could be determined by hybridising Mu fragments with varying concentrations of *E. coli* DNA. The *c* end and the *S* end bands were cut out from the nitrocellulose papers after hybridisation and read in a scintillation counter. *E. coli* DNA always hybridised with the *S* end 10–20-fold more than with the *c* end. Since the mean length of the *S* end heterogeneous sequences is about 1.5 kb (see ref. 6), it follows that the *c* end generally has 75–150 base pairs of heterogeneous host sequences.

Fig. 3 Hybridisation with *E. coli* DNA to Mu fragments produced by *Hind*III, *Eco*RI and *Hind*III + *Eco*RI. Mu *c3* DNA fragments visualised after ethidium bromide staining are on the left. Autoradiograph of the same fragments after hybridisation with ³²P-labelled *E. coli* DNA is on the right. The experimental procedure was as in Fig. 2 except that electrophoresis was carried out in 0.9% Agarose gels to resolve large fragments. In each case the most rapidly moving band represents the *c* end and the second band from the top represents the *S* end. In *Eco*RI digest a partial fragment, because of incomplete digestion, can be seen at the top. The marker DNA was a mixture of labelled Mu fragments and an unrelated labelled fragment. The unrelated marker fragment can be seen as a dark band in the autoradiograph. The fragments and their pattern of hybridisation are diagrammatically shown in the insets at the bottom. With *Hind*III and *Eco*RI, the middle fragment of Mu did not show significant hybridisation with *E. coli* DNA, whereas both the *c* end and the *S* end fragments did. With *Hind*III + *Eco*RI digestion, out of 5 fragments (see map in Fig. 2) only B (*S* end) and E (*c* end) hybridised with *E. coli* DNA.

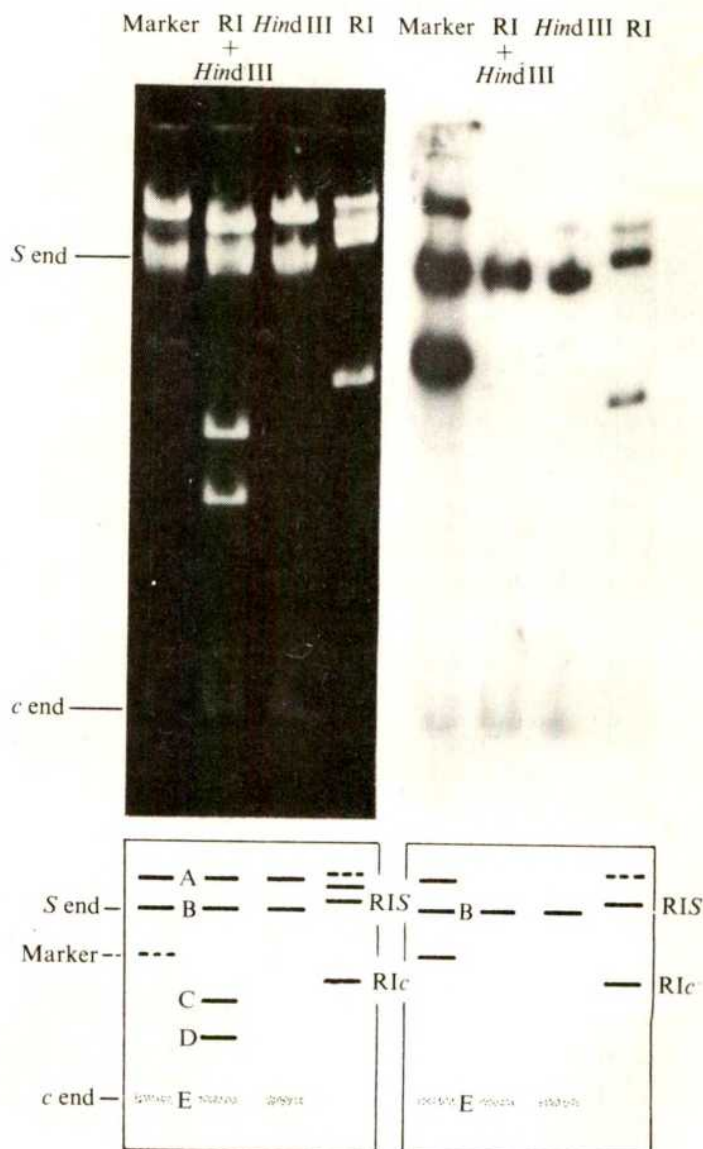
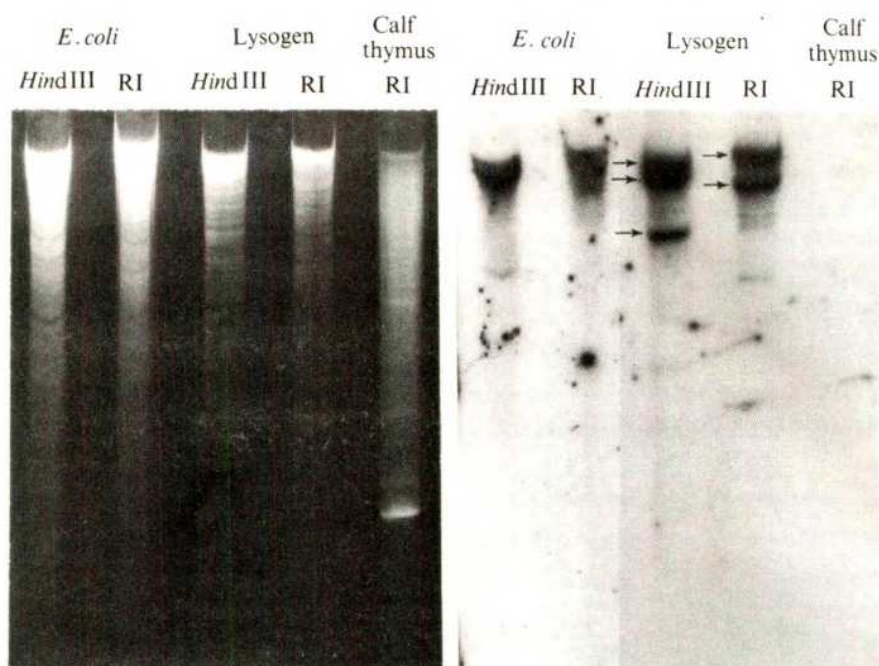


Fig. 4 Hybridisation with Mu DNA to fragments of *E. coli* DNA. Total cellular DNA from KL16, an *E. coli* K12 strain, and from a Mu *cts62* lysogen was extracted three times with phenol and digested with *Hind*III or *Eco*RI. Calf thymus DNA, digested with *Eco*RI, was used as control to check for stringency of hybridisation. Mu DNA was labelled *in vitro* with ^{32}P to high specific activity by the so-called "nick translation technique", as described by Maniatis *et al.*¹⁵. DNase I (1 ng) was added to cause nicks and then the nick translation reaction carried out with DNA polymerase using ^{32}P -labelled nucleotide triphosphates. The DNA bands visualised after ethidium bromide staining are on the left. The brightly stained bands in the calf thymus DNA column are comprised of tandemly-repeated sequences that are represented many times in the DNA complement. No hybridisation of Mu DNA with calf thymus DNA can be seen in the autoradiograph on the right. Hybridisation with *E. coli* DNA can be clearly seen at the top; the intensity of bands in the autoradiograph decreases as fragments become smaller and smaller towards the bottom of the gel. The *Hind*III digest of DNA from a lysogen (BU 1209) shows three prominent bands whereas the *Eco*RI digest of the lysogen DNA shows two very strong bands after hybridisation. These bands, indicated by arrows, represent the prophage Mu DNA. The principle for detecting the integrated Mu genome was as described by Botchan *et al.* for determining the organisation of the simian virus 40 DNA in rat cells transformed by SV40. In the *Eco*RI digest of the lysogen DNA, the top band represents the middle part of the prophage genome and the *S* end junction fragment of prophage and host DNA. The second band represents the *c* end junction fragment. In the *Hind*III digest of the lysogen DNA the top two bands (not completely resolved) represent the middle fragment and the *S* end junction fragment. The third band is the *c* end junction fragment. The size of the end fragments has changed because of fusion with host DNA. The significance of the minor discrete bands in *E. coli* DNA digests, which can be seen after hybridisation with ^{32}P -labelled Mu DNA, remains unclear.



These results provide direct proof that *E. coli* sequences are present at both ends of Mu DNA and that these sequences are picked up from different parts of the chromosome of *E. coli*. The host sequences at the *c* end correspond to 75–150 base pairs. These sequences, as opposed to the *S* end sequences, are generally too short to be seen by electron microscopy of denatured and renatured Mu DNA molecules. It is possible, however, to see them electron microscopically by binding T4 gene 32 protein to the single strands (H. Delius, personal communication).

The host sequences at the *S* end have been postulated to arise by headful packaging of Mu DNA from maturation precursors in which Mu DNA is covalently linked to host DNA. This would mean that the headful packaging of Mu

starts from the *c* end, linked to host DNA, and proceeds toward the *S* end, also linked to host DNA and that the presence of host DNA at the *S* end is a phenomenon of the size of Mu DNA. Experimental evidence has confirmed that insertions in Mu DNA cause a reduction in the length of the *S* end host sequences, as expected from the headful packaging model.

The *X* mutants of Mu, containing an ISI type of insertion of about 800 base pairs¹⁴, show shorter single-stranded tails on denaturation and renaturation⁶. Insertion of a 2.8 kb long translocation element, carrying gene for chloramphenicol resistance, completely eliminates the *E. coli* sequences at the *S* end (Chow and A.I.B., unpublished). Insertions in Mu seems, however, to have no effect on the

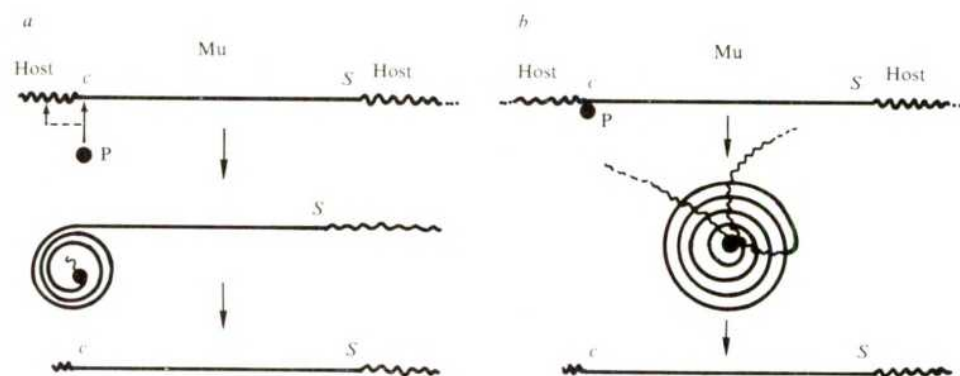


Fig. 5 Generation of *E. coli* DNA at the ends of Mu. Two possible models shown are based on the assumption that the maturation precursor contains *E. coli* DNA linked to both ends of Mu DNA. *a*, P recognises a specific site at the *c* end but cuts to the left of the prophage DNA, into the host DNA. The DNA is then folded until a headful is reached at the *S* end. *b*, P recognises a specific site at the *c* end and serves as a nucleation centre for condensing DNA. When a headful is reached, cuts in DNA are made. The cut into *E. coli* DNA near the *c* end is made because the nucleation centre is large, covering a part of the host DNA at the *c* end. The representation is strictly diagrammatical.

size of the host sequences at the *c* end. The *c* end fragment produced by *Hind*III remains diffuse on gels in the case of both small and large insertions in Mu DNA (data not shown). The acquisition of 75–150 host base pairs at the *c* end is apparently a specific event, unrelated to the size of Mu DNA being packaged. Two possible ways in which a small amount of host DNA can be linked to the *c* end of viral DNA are depicted in Fig. 5. It can be postulated that the 'packaging proteins' recognise a specific site, very close to the *c* end of the Mu DNA, cut the DNA to the left of the recognition site and then fold the DNA until a certain size is reached. This would imply an interesting endonucleolytic cut by Mu proteins. Alternatively, the DNA could be condensed by a large packaging complex which covers a part of the host sequences linked to the *c* end such that when the packaged DNA is cleaved from the maturation precursor some host DNA is retained at the end. In any case, cutting into host sequences at the left, and also to the right, of the actual Mu DNA ends ensures that Mu sequences remain intact during the viral life cycle.

Mu is the only viral system known so far in which viral DNA acquires new host sequences during maturation. The normal presence of host DNA at phage ends thus has no precedent. The temperate bacteriophages generally have either cohesive ends or have duplicated end sequences which are used to efficiently convert the linear DNA molecules into circular forms. Bacteriophage Mu falls into a third category. It has heterogeneous sequences at the ends of its DNA and has no obvious means of fusing them to form circular molecules.

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Photoformation of polychlorinated biphenyls from chlorinated benzenes

In recent years there has been considerable public concern about polychlorinated biphenyl (PCB) residues in the environment. Many studies have indicated the main causes and the present problems with regard to the contamination by PCBs^{1–6}. Little attention has, however, been given to the possible presence of PCBs in many organic chemicals. We report here the formation of PCBs from some chlorinated benzene compounds by sunlight irradiation, and show that these PCBs have different gas-liquid chromatographic (GLC) profiles from the industrial PCBs.

The following reagent grade chlorobenzenes used as

Table 1 Photoformation of PCBs from chlorinated benzenes*

Chlorinated benzenes	Irradiation (d)				No. of Cl atoms calculated
	0	14	28	56	
MCB	5.5	690	1,060	NA†	1
<i>o</i> -DCB	1.4	940	970	2,270	3
<i>m</i> -DCB	1.1	340	350	520	3
<i>p</i> -DCB	0.7	310	340	1,860	3
1,2,3-TCB	0.7	29	32	32	5
1,2,4-TCB	4.0	5,710	8,470	9,770	5
1,3,5-TCB	1.7	56	98	160	5
1,2,3,4-TECB	2.4	680	1,200	4,280	7
1,2,4,5-TECB	4.0	9	26	NA	7
HCB	1.0	36	41	64	5

*p.p.m.

†NA, not analysed.

materials were purchased (Wako Pure Chemical Industries, Ltd, Osaka, Japan); monochlorobenzene (MCB), *o*-, *m*-, *p*-dichlorobenzene (DCB), 1,2,3-, 1,2,4-, and 1,3,5-trichlorobenzene (TCB), 1,2,3,4-, and 1,2,4,5-tetrachlorobenzene (TECB), and hexachlorobenzene (HCB). Twenty grams of each of these were placed in 100-ml Hario brand borosilicate glass-stoppered Erlenmeyer flasks (Shibata Chemical Apparatus Co., Tokyo) which were stoppered and irradiated outdoors in sunlight for 56 summer days in Kochi, Japan.

The separation of PCBs from chlorobenzenes was carried out as follows: 0.1 g of an irradiated chlorobenzene was dissolved in 2 ml hexane, placed on a 20-g, 19-mm interior diameter silica gel column and eluted first with 250 ml hexane which removed MCB, DCBs, TCBs, TECBs and HCB, and then with 300 ml methylene chloride-hexane-acetonitrile (80:19:1) which eluted PCBs. The second fraction was cleaned up with a 10-g Florisil column using 200 ml hexane as eluting solvent. Average recoveries of PCBs in this separation process were 96.9% when the sample has fortified at 100 p.p.m. with KC-500, an industrial PCB.

The quantitation of PCB residues has usually been done by measuring the total peak height of the electron capture GLC response for residue against that of industrial PCB such as KC-500. We did not determine PCBs formed from chlorinated benzenes by the above method because their GLC profiles were not similar to those of industrial PCB. The fraction containing PCBs was perchlorinated using the method described previously^{7,8}. Decachlorobiphenyl formed by perchlorination of a mixture of PCBs was easily detectable as a single peak by electron capture GLC using 1% SE-30 column at 210 °C. The residue values were determined based on the electron-capture response to the decachlorobiphenyl compared with the standard, and then calculated so as to express the amount of PCBs containing an equivalent number of chlorine atoms.

PCBs were determined in all irradiated chlorobenzenes throughout the experimental period. GLCs showing PCB photoformation from a number of chlorinated benzenes are presented in Fig. 1, and the amounts formed are given in Table 1. A single peak due to a substance containing one chlorine atom was found to originate from MCB; PCBs containing three chlorine atoms were photoformed from DCBs; PCBs formed from TCBs almost always contained five chlorine atoms (at least six PCBs were observed in products from 1,2,4-TCB compared with only one PCB formed from 1,2,3-, and 1,3,5-TCB). Several PCBs containing seven chlorine atoms were formed from each of the TECBs; PCBs formed from HCB were found to have four or five chlorine atoms; GLC-MS suggested that the chemical structure was dimethyl chlorobiphenyl but it is unclear where the methyl groups originated: the problem is under investigation.

The reaction mechanism of photoformation of PCBs may

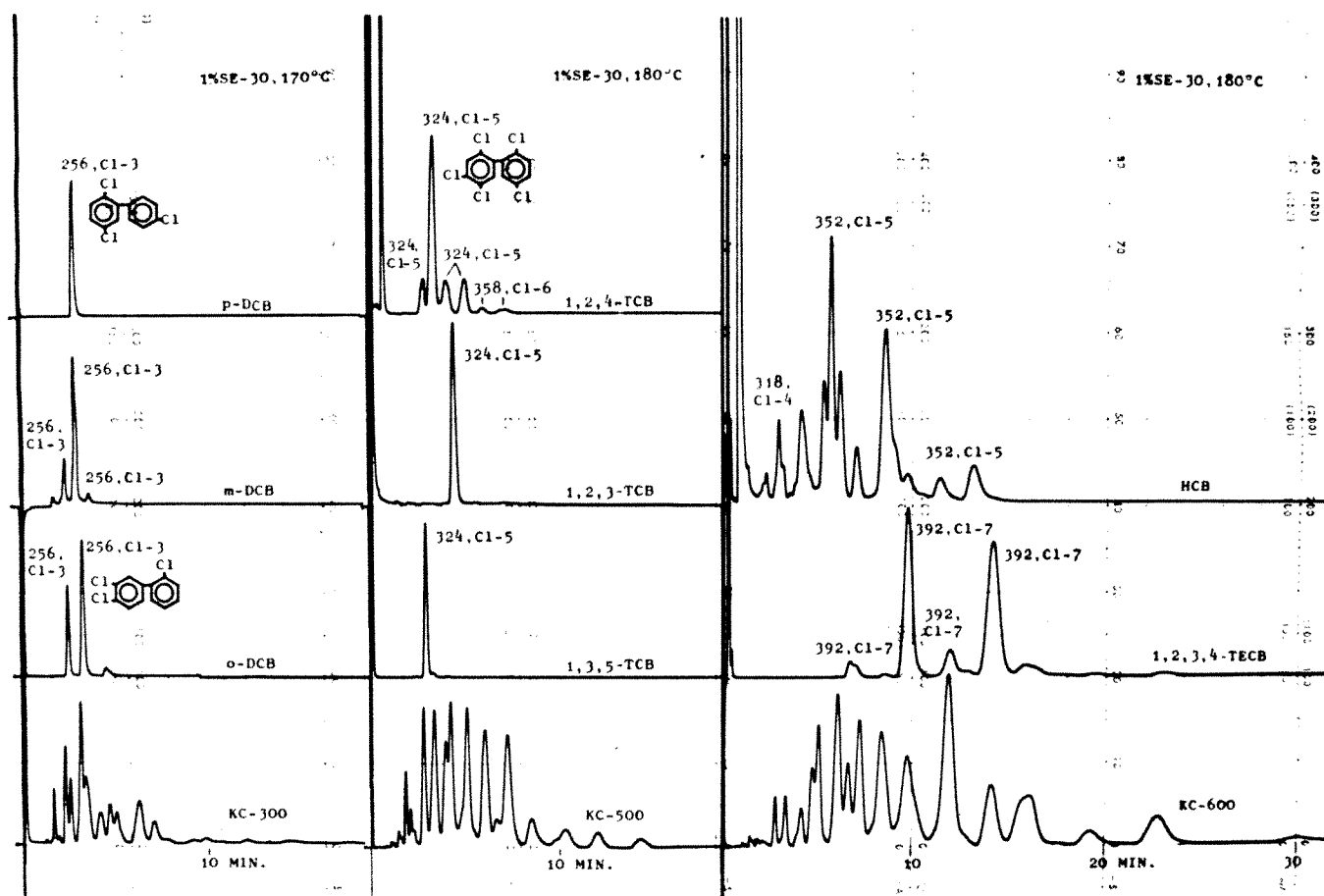


Fig. 1 Electron capture gas-liquid chromatograms of PCBs formed from chlorinated benzenes by sunlight irradiation. The KC series are Kanechlor, industrial PCBs (Kanechlor Chemical Industries Co., Ltd, Osaka). Each peak was determined by GLC-MS and both the GLC peak position and the *m/e* of the molecular ion are shown together with the chlorine content. The chemical structure of substances causing the main peaks of PCBs have been proposed by comparison with GLC reference standard of known chlorinated biphenyls made by Analabs Inc., Connecticut.

involve free radical reactions based on the dehydrochlorination of one molecule from two molecules of the parent chlorobenzene. This is supported by the following: (1) the formation of hydrochloric acid in the reaction mixture; (2) the number of chlorine atoms in PCBs formed by irradiation was one less than those contained in two molecules of the equivalent parent chlorobenzene; (3) PCB formation was remarkably inhibited when the radical scavenger, DPPH(2,2-diphenyl-1-picrylhydrazyl) was added to a chlorobenzene, and the amount of PCB formed decreased to approximately one hundredth in the case of 1,2,4-TCB. Conversely, an increase in photoformation resulted when photosensitizer, benzoyl peroxide, was added, the amount of PCB formed increasing threefold in the case of 1,2,4-TCB; (4) in addition, it is possible to deduce theoretically that 2,5,4'-trichlorobiphenyl is formed from *p*-DCB and 2,4,5,2',5'-pentachlorobiphenyl from 1,2,4-TCB.

Although each chlorinated benzene has strong ultraviolet absorption in the ranges 210–230 nm and 250–300 nm, the borosilicate glass container prevented sunlight at wavelengths less than 300 nm from entering. Therefore, it was impossible to investigate the possible effect of the ultraviolet component of sunlight in these experiments, but PCB formation from chlorinated benzenes under irradiation by ultraviolet light is now being studied.

Previous workers^{9,10} have indicated that some PCBs are products of DDT photolysis. This paper demonstrates that appreciable quantities of PCBs are also formed photo-

chemically from other types of synthetic organochlorine compounds. We suggest that caution should be exercised when manipulating chlorinated benzenes industrially and when handling household insecticides such as *p*-dichlorobenzene.

We thank S. Takahashi (Shimadzu Seisakusho Ltd) for GLC-MS and L. Kovach (JAOAC editorial office) for suggestions in the preparation of this paper.

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Christmas

books

supplement

Colour, song and movement

Peter Conder

BIRDS probably command a greater following at many levels of interest throughout the world than any other group of wild animals or plants because they are easily seen and recognised. Their colours, songs and movements fascinate, and their ways of life provide an interest for a growing number of serious bird-watchers. The books reviewed here reflect some of the different levels and areas of interest in birds: one relates facts about the adaptations of birds to their habitats in various parts of the world; and five others deal with more discrete groups. None of the books is highly technical.

I tend to be suspicious of large 'coffee-table' books which consist of glossy photographs or paintings often supporting a poor text. In *Birds and their World* (Hamlyn: London and New York, £2.95), however, John Andrews has written one of the best texts that I have found in such surroundings. Lucidly written, and full of facts about the relationship of birds to their habitat in the major biomes of the world, the book describes structural and functional adaptations to the particular characteristics of the biomes. A single chapter on migration seems out of place. As one might expect, since it is a Hamlyn publication, three-quarters of the book are taken up by colour photographs. These are of ex-

cellent quality generally, but a few have been enlarged far too much. At £3.95 it is good value for the general bird-watcher.

More expensive and less value for money is *Penguins: Past and Present, Here and There* (Yale University: New Haven and London, £6) by George Gaylord Simpson, Professor of Geosciences at the University of Arizona. Simpson became interested in penguins when, in the course of field research, he discovered a collection of fossil penguin bones. Despairing of finding any palaeo-ornithologist to work on the collection, he embarked on the task himself. In his book, therefore, the most interesting and worthwhile chapter is written round his own discoveries. Since that study he has visited many penguin colonies but has not been able to undertake further research. The remainder of this book therefore relies on previously published literature, of which he gives a limited bibliography. It is a pleasant enough "informal account" to quote the book jacket. The black-and-white photographs have not reproduced too well and the colour photographs are amateurish.

Very different is the contribution of the author in two books on raptors—*Birds of Prey: Their Biology and Ecology* (Hamlyn: London and New York, £4.50) and *Eagles of the World* (David and Charles: Newton Abbot and London, £4.95). Nominally they are put out by two different publishers but the page design, type and binding are

extraordinarily similar. In both books Leslie Brown reviews what is known about various aspects of the life of raptors—classification and distribution, habitats, way of life, way of hunting, breeding biology, ecology—and adds much original material. There is some overlap between the books, and in *Birds of Prey*, the author inevitably writes in greater detail on eagles. In both books he points out areas in which research is needed and could be easily undertaken. The books are rather discursive in style but interesting in an anecdotal sort of way. Both books are illustrated by explicit diagrams and line drawings; black-and-white photographs only in *Eagles of the World*, but both colour and black-and-white photographs in *Birds of Prey*. Each is provided with a fairly large "select" bibliography, very useful appendices summarising a variety of data. Both are good value for money.

Winter Birds (Michael Joseph: London, £5.75) by Malcolm Ogilvie concentrates on those birds that live and breed within the Arctic Circle. Aimed at readership on both sides of the Atlantic, Malcolm Ogilvie begins his book with a chapter describing the nature and extent of the Arctic followed by another on the adaptations by birds to Arctic conditions. Four chapters follow on waterfowl, sea, shore and land birds found in the Arctic, and the book finishes with a chapter on the need for conservation in the Arctic. Generally the text maintains a high level of interest but I

Above illustration: *The Archangel Gabriel, part of a miniature from Syria or Egypt (14th century, British Museum, London). Taken from Islam and the Arab World, reviewed on page 596.*

wondered if giving each bird species found in the Arctic was the best way of dealing with this group of birds. Some accounts are interesting, particularly those on waterfowl, which are the author's speciality; and on waders, such as knot, on which much research has recently been carried out. But some other sections are mere repetitions of textbook material. Chapters describing and comparing the characteristics of individuals of the group in the Arctic might have been far more readable and have provided an opportunity for more discussion. Maps and photographs are useful additions to the text as is the up-to-date bibliography. The book is well produced—recommended for Arctic enthusiasts.

Tony Soper's book *Everyday Birds* (David and Charles: Newton Abbot and London, £2.95)—a very popular account of the lives of nine very common birds—will please children and the less discriminating adult bird lover. Much of the text is very simple, factual and moves at a great pace, which is possibly why he makes the occasional careless statement such as "birds are controllers of insects" and "birds have a great sense of adventure". This book will be lapped up by a large market who will also enjoy Robert Gillmor's excellent drawings. □

Peter Conder has recently retired as Director of the UK Royal Society for the Protection of Birds.

Spirit of Nature needs rekindling

OVER the past ten years, the study of animal social behaviour has undergone a dramatic change. Gone are the days when animals were held to do things 'for the good of the species' or for the benefit of their group if it meant that they themselves suffered in the process. Altruism—in the sense of behaviours which benefit the reproductive success of other unrelated individuals at the expense of an animal or its relatives—is contrary to the Darwinian theory of natural selection. Selfishness and exploitation are the rule. So harsh a master is natural selection that it is not even the individual that benefits in the long run, but his genes. The individual animal has a finite lifetime, but his genes, in the bodies of his offspring, and in the offspring of his brothers and sisters or other relatives live on. Hence any genetic propensity to benefit relatives will be increased because the relatives will also be likely to have the same propensity. This idea of 'kin selection' has been fully developed by W. D. Hamilton.

In view of this shift of thought ('revolution' is too strong a word: it is more a realisation of what was implied all along by the theory of natural selection), it is disappointing to find that so many recent popular books still perpetuate the fallacy that the species is the unit of selection and seem unaware of recent developments in the field of evolutionary biology. This is particularly apparent in *The Mating Game* (Elsevier-Phaidon: Oxford, £4.50) by Robert Burton, a lavishly illustrated account of animal courtship and reproduction. The photography is most impressive, but the text is full of such statements as (p10) "the individual is unimportant and survives solely for the benefit of the whole", and (p8) "the

function of sex is thus to ensure that animal populations are composed of a variety of individuals". The function of sex is one of the most hotly debated issues among evolutionists precisely because 'good of the population' arguments have been found to be inadequate. It may certainly be advantageous for the population as a whole to be variable, but the problem is that sexual reproduction involves producing individuals that contain only half the genes of each parent, an apparently enormous cost genetically, which asexually reproducing individuals would not have. The work of R. L. Trivers has made us take a new look at animal courtship and pair bonding. Animal family life is seen as a world of exploitation of one sex by the other, of cheating and of strategies involving cooperation only when this is selfishly the best thing to do. There is no mention of this aspect in the book, nor any of the genetic relationships between the members of a social insect colony, which is crucial to an understanding of their curious reproductive system.

Something of the same fallacy is apparent in John Napier's book *Monkeys without Tails* (BBC: London, £5.25), which on p33 states that "the whole life style of animals is a preparation for ensuring the continuity of their species" and that "animals that do not reproduce are biologically irrelevant". On the contrary, kin selection theory has shown that non-reproducing individuals may be genetically very important. This book, although a very interesting account of primate biology does not quite live up to its subtitle—*A Giraffe's-eye View of the Evolution and Life History of Man and the Chimpanzee*. The idea is an appealing one—to look down on man from a great height as a rather ordinary primate—but the book is nevertheless rather noticeably anthropocentric. Man is placed at the top of the primate

"staircase", with the "great divide" separating us from our primate relatives. There is a lot of useful information in this book, however, and it is very readable.

The Secret Life of Animals (Weidenfeld and Nicolson: London, £8.95) by Lorus and Margery Milne and Franklin Russell also insists that (p112) "whatever the behaviour is that best suits the survival of the species, occurs", but is redeemed by some really superb photography. The book really does succeed in conveying a sense of awe and wonder about animal life, which *The Amazing World of Animals* (Thomas Nelson: London, £4.50) edited and with a foreword by Sir Peter Scott also sets out to do. This book contains some good photographs, and chapters on different animal groups by various authors.

The Classification of Animals (White Lion: London, New York, Sidney and Toronto, £3.75) by Richard Freeman is a most useful illustrated summary of the classification of living animals, with examples, a clear indication of the numbers of species in each group covered, and a brief but informative description. It is concise, but at the same time satisfyingly comprehensive—the basic taxonomic information needed by a zoologist in a few pages.

The Language of Smell (Routledge and Kegan Paul: London and Boston, Massachusetts, £3.95), also by Robert Burton, is a summary of how animals smell and the uses to which they put their sense of smell, at a fairly elementary level. The recent evidence that shows that von Frisch has been right all along in claiming that the dances of honeybees transmit directional and distance information, is apparently unknown to the author. One is therefore, mistakenly given the impression that von Frisch is now generally considered to have been wrong and that bees rely on smell alone.

It is a relief to turn to *Bird Life: An Introduction to the World of Birds* (Elsevier-Phaidon: Oxford, £5.95), with a text by Christopher Perrins. Here at least the basic evolutionary framework is sound and the current thought on social behaviour, mating, and so on, is simply and well explained. The book covers in a readable way various aspects of bird life, such as feeding, social behaviour, migration and so on. It is clear, non-technical and has numerous coloured drawings by Ad Cameron, which although expert and lifelike, might perhaps be thought a little monotonous as the sole source of illustration.

Marian Dawkins

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Capturing the secrets of Nature

NATURAL HISTORY must still be the subject most likely to kindle an interest in science in the tinder-dry mind of a young child. The problem is how to tend the flame so that the interest becomes a passion. When I was a child the process included Nature walks, listening to BBC radio's *Nature Parliament* and *How Things Began* and, of course, books. Now, streets ahead of anything else, there is film.

Film allows us and our children, at home and at school, to view every intimate detail of the erstwhile secrets of Nature. No longer do we wonder what goes on in the darkness of a woodpecker's nest nor contemplate the riddle of the landing of a fly on the ceiling. Given a steady diet of fantastic film, can the jading palate of today's child still be tempted by a book on Natural History? To judge by the masses of such books on the market the answer must be yes, or at least the adults who buy the books must believe so. For a book to be successful, however, it must surely attempt to match rather than ignore the dramatic qualities that the moving film has brought us. Only one of three series currently available really does that.

The success of the *A Closer Look At . . .* series (Hamish Hamilton: London, £1.50 each) rests heavily on the illustrations and the consistently good, sometimes inspired, layout. There are no photographs in these books. Instead the pages are generously supplied with full colour drawings which, at their best (for example, in . . . *Elephants* and . . . *Apes*) are vivid double-page spreads capturing a group of animals in mid-action. Each double page also has a few hundred words of text addressed to a particular topic, which vary from book to book but which generally start with evolution or geography and close with the prospects for . . . Information in the main part of the book is, however, centred either on species or behaviour. With such a limited amount of text the choice of topics or facts has inevitably been slightly arbitrary but a certain balance has nevertheless been maintained in each volume.

The publishers of the series make no claim as to the age group for which it is suited. The books certainly succeed as picture books for the six year old—particularly the more gory offerings, such as the death by leopard of an Australopithecine boy (. . . *Prehistoric Mammals*) or the spider-killing wasp (. . . *Bees and Wasps*) and I am sure that the text would carry the series up to the start of the teens. There are occasional oddities: the concept of



Emperor tamarin (marmoset monkey)

ridiculous and ridiculous birds may be worth a page in the . . . *Birds* book but surely one could do without the terms themselves. And failings: the quality of the illustrations in . . . *Arctic Lands*. But overall these are excellent and reasonably priced books.

The second series, to which *How Insects Live* (Elsevier-Phaidon: Oxford, £4.50) is the latest addition, is clearly, if not explicitly, aimed at the teenager. But it would have to be an already committed one who actually benefited from the books. That, to my mind, is the failing of the series. In general design the volumes resemble a 'coffee-table' production both in size and in the abundance of handsome colour photographs. But the design belies the text which is by turns dull, didactic or of the "just-fancy-that" variety. Where

exactly did the editor expect to find readers whose attention would be captured by Haeckel's dictum that "ontogeny repeats phylogeny" (*How Fishes Live*, £3.95) or by the correlation between the breeding behaviour of ring doves and the weights of their oviduct and crop contents (*How Birds Live*, £3.95)? Perhaps the bookish student, aged 15 or over, will appreciate these volumes; otherwise, buy them only if you cannot resist the photographs.

The *Nature's Way* series (G. Whizard/André Deutsch: London, £1.75 each) is to my mind an even greater failure. The origin of this series is the Oxford Scientific Film Unit which is rightly renowned for its Natural History films. These books (*Bees and Honey*, *Life and Times of the Stickleback*, *Butterfly Cycle*) are clearly spin-off material. Each consists of about five pages of simple text followed by about twenty full page coloured photographs. The text is unexceptional, and the photographs are generally fine, but I was dissatisfied, perhaps through frustration, at the inevitable failure to translate action into stills photography. Once through these books was enough for me, and would, I suspect be enough for children. Not enough, however, to be able to recommend them as worth buying.

I have surprised myself by coming down heavily in favour of the series of books that relies on artistic rather than photographic illustrations. If you want the 'real' thing I can only say stick to television or, far better, head for the country.

Peter Newmark

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Nature on the seashore

ALTHOUGH books about the seashore might seem more relevant as a preparation for summer holidays, they may nevertheless make acceptable Christmas presents for scientists specialising in other subjects, laymen or the generation still at school or university. Two books have appeared in the shops during 1976 and it is interesting to compare them with some other books written over the last 27 years which are still available.

Taking the new books first, we have *The Hamlyn Guide to the Seashore and Shallow Seas of Britain and Europe* by A. C. Campbell (Hamlyn: London and New York, £2.25). This book is bound in durable paper that might withstand rain or seawater to a limited extent during shore collecting. The illustrations, by James Campbell, all in colour, are well above the average for this type of book. After a very brief

introduction on types of seashore, tides and collecting, the non-biologist is thrown in at the deep end with two pages of "key" which cover the entire plant and animal kingdom. The reader must then find his or her way to the right phylum where a brief description of the phylum is followed by a list of species divided into classes and families. It is essential for the reader to find the plant or animal by its picture. Once this has been achieved the genus, species, authority, popular name, size, brief description, habitat and distribution are given. At the end of the book there is a glossary, bibliography and index. For identifying shore life, this book may well be the best available on the market and at its modest price is strongly recommended for the amateur shore collector.

The second new book, *A Field Guide to the Mediterranean Seashore* by W. Luther and K. Fiedler, translated and edited by P. J. Miller (Collins: London, £4.95) is a hardback with a cover of what seems to be

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waterproof material. After a somewhat longer introduction on habitats, each phylum is described in turn and its representatives given by class, order and family. Although each species is briefly described with Latin and popular name, size and habitat, the illustrations are much poorer than the Hamlyn book; many are only black-and-white, and those in colour are given against a coloured background that seems to provide an unnecessary and sometimes confusing contrast. The book ends with a section on collecting, marine aquaria, a bibliography and index. There is no glossary or general introductory key so the non-biologist, although he might find the book a useful companion on a Mediterranean holiday, may have difficulty in tracking down a specimen.

Collins' Pocket Guide to the Seashore by J. H. Barrett and C. M. Yonge (Collins: London, £3.95) is still being reprinted as the 1958 edition in hardback. Covering both plants and animals, it is divided into phyla and the main subgroups (down to species) to be identified by a proper key. Having at last arrived at the species, the Latin and popular names are given, with a fairly full description of anatomy, habitat and distribution. This is a more difficult book for the amateur (or

professional) to use; not all species are illustrated; and the illustrations (partly black-and-white, and partly coloured plates of good quality) are placed together near the centre of the book and not with the species description. There is a bibliography, index and a useful introduction. According to the introduction the book is aimed at amateur naturalists, who will probably find more species described than in the Hamlyn book but will certainly require more patience in locating their specimens from 'first principles'.

In very different style is *The Seas* by F. S. Russell and C. M. Yonge (Warne: London and New York, £6.95). First published in 1928 and reviewed in its revised form by Sir Cyril Lucas (*Nature*, 258, 36, 1975) we find a book divided into 17 well-illustrated chapters. This is not a book for identification or reference; it is a collection of essays in non-technical language on various aspects of marine biology. The book is readable, and would provide the amateur with a good general picture of all aspects of the sea and sixth-formers or first-year university students with worthwhile background information.

Equally well-produced is *The Sea Shore* by C. M. Yonge (Collins: London, £6.50 hardback, £0.95 paper-covers). This is one of the celebrated New Naturalist Series originally published in 1949, reprinted last in 1971

and still available. It is at a similar technical level to *The Seas* but the 19 chapters are restricted to the shore areas, with a more detailed description of subjects like Life in Rock Pools, Zonation on Rocky Shores, and Life on Sandy Shores, Mud or in Estuaries. The book is beautifully illustrated and highly readable and will take a rightful place with others of the New Naturalist Series on any bookshelf.

Finally, *Fishes of the Sea* by J. N. and G. Lythgoe (Blandford: London, £3.90 hardback). Although described as a coloured photographic guide to the fishes of the British Isles, Northern Europe and the Mediterranean, it consists also of an introduction to fish anatomy as well as descriptions and line drawings of each species. There are many coloured and black-and-white underwater photographs placed together at the centre of the book which might be used by SCUBA divers for identifying fish in their natural surroundings. The book is thus of restricted value to the shore collector and non-diver, but would be a worthwhile addition to the luggage of amateur divers holidaying next summer either in the UK or on the continent.

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Enthusiasm for shell collecting

The Shell Collector's Guide: An Introduction to the World of Shells. By S. P. Dance. Pp. 192. (David and Charles: Newton Abbot and London, August 1976). £4.95.

SHELL COLLECTING or conchology is among the oldest known intellectual leisure activities. The fascination of "shells that speak seven seas" (one of the many apposite quotations from unlikely sources—this one is from Dylan Thomas) is evidenced by shell jewellery from archaeological sites about thirty thousand years old. More recently, excavations at Pompeii have unearthed shell collections, evidence of the interest in conchology that this book will doubtless help to perpetuate. Peter Dance has written an enthusiastic, honest, technically expert and enjoyable book about a semi-scientific hobby which can bring great contentment (and occasional heady excitement) to young and old alike.

Many naturalists have tried to put into words something of the fascination of conchology. As a hobby, it is all

things to all men. It appeals to the outdoor person who wants to punctuate his rambles by rustic searches; to the SCUBA diver and his less adventurous wading or dredging comrades; to the young who marvel at the exquisite geometry of shells; to the old who take satisfaction from their patient systematic accumulations over the decades and from postal exchanges with enthusiasts all over the world; to those of an artistic persuasion who love the beauty of shells; to scientists who yearn to make order from chaos and enjoy arranging, naming and cataloguing their collections. In short, first and foremost, conchology is FUN. It can, and occasionally does, lead to advances in the scientific study of the Mollusca, and can bring rich intellectual rewards to those who embrace it. It can also be physically pleasant to handle shells, although not everyone would agree with Mr Dance that touching cowrie shells (for example) provides a "decidedly sensual tactile satisfaction".

This book will tell you everything you need to know to begin searching for, cataloguing, preserving and displaying a shell collection. It tells you about shell clubs, shell shows, valuing,

exchanging and buying shells. Fascinating sections of the book deal with shell frauds, and with the commercial exploitation of the rarest shells. Did you know, for example, that prices of certain shells at auctions have now topped the \$4,000 mark?

One of the best features of the book is the high quality of the illustrations, with a splendid series of photographs by Ian Cameron and well chosen and beautifully executed drawings by Annabel Milne. The standard of production of this book is uniformly excellent and it is pleasant to note that it was printed and assembled entirely in Britain.

Why then is my brow furrowed? Simply because I share with many other professional zoologists a frightful vision of a world in which all the molluscs are on display in skilfully designed cabinets, and the seas, lakes, marshes, streams and woods are malacologically empty. If anyone doubts that this is more than an irrational nightmare, he should ask himself how many butterflies he has seen in Britain during recent years; have lepidopterists any share of the blame for their dearth?

The author offers no balm for my anxieties about the possible predatory effects of indiscriminate shell collecting. He advocates searching for living molluscs (so that they may be killed for their shells) rather than taking empty shells from the beach deposits, and describes in detail various unpleasant methods of torturing the animals out of their shells after capture. He describes one method which is so hideous that he says he never himself has practised it (why then lend it currency?).

About wholesale commercial conchology (reef raping, as an Australian described it) he lamely says he does not propose to take sides or to argue the rights and wrongs. About the legitimate and calmly voiced fears of naturalists about the future of the mollusc world, wanting to keep the word 'conservation' before the shell collector, Mr Dance says only this: "For some mysterious reason, the scientific world appears to think that it has almost a divine right to Nature's products". This is unfair. Speaking personally, I do not want to conserve molluscs simply so that I and others in my laboratory can catch and experiment on them; I want to conserve them so that my grandchildren (and Mr Dance's) can see and admire them. I hate to feel that I may be living in the last generation of mollusc enthusiasts.

T. E. Thompson

T. E. Thompson is a Reader in Zoology at the University of Bristol, and President of the Malacological Society of London, UK.

Artistic impetus for scientific observation

Bright Wings of Summer: Watching Butterflies. By D. G. Measures. Pp. 160. (Cassell: London, October 1976.) £5.

THIS book is the work of an artist. David Measures goes out to his disused railway line in Nottinghamshire and draws and paints, describing what he sees in the butterfly world at various times of the year and in different weather conditions. He reproduces his notes and his delightful sketches, full of movement and swift life, and in the past twelve years he has collected a great deal of first-hand information on topics such as scents, courtship, mating, egg-laying and territorial boundaries in a unique way which is not to be found in the standard books.

The object is to set out the life histories and behaviour of butterflies in a way which will stimulate readers to become interested in Natural History and to make and record their own accurate observations. The unfinished paintings and the roughly written notes show how in practice conclusions are arrived at.

The book is divided into three parts. In "The Beginnings" he describes his own life history. He started with a general love of nature and then, because of his preoccupation with colour, concentrated on butterflies. "There is nothing so brilliant, or as changing and shifting as the iridescence and the pigmented colour of the butterfly's wing".

Part 2 gives the results of his observations, and lastly, "You and

Butterflies" gives practical advice as to how to follow in his footsteps.

The lesson for the pure scientist is that each insect is an individual and that, when one classifies, something is lost—although for a physician-reviewer accustomed to looking after patients this does not come as a surprise.

On the other hand, art must not be allowed to get away with it completely, and with more scientific knowledge the author's observations could have been more productively channelled. What precisely happens to our red admirals in the autumn? Burnet moths are resistant to cyanide, and cinnabar caterpillars feed on the poisonous ragwort—what are the predators of these insects? Do the black and white forms of the peppered moth actually settle on appropriate backgrounds? Similarly, where in nature do caterpillars with brown and green chrysalids actually pupate?—the colour and texture of the site are of great interest. The extent of bat predation at dusk also needs investigating.

Other criticisms are that there should be more emphasis on the difference between camouflage and warning coloration (p. 72) and surely the plate opposite page 100 is not the small heath. In general, however, the photographs are superb.

The book can be strongly recommended for nature lovers with artistic inclinations, but there is no reason why imagination and science should not be brought closer. E. B. Ford's *Butterflies* (which is recommended for further reading) exemplifies what I mean.

Cyril A. Clarke

Sir Cyril Clarke is a general physician with an interest in genetics which stemmed from breeding butterflies. He is President of the Royal College of Physicians.

Jumbo the elephant

Jumbo. By W. P. Jolly. Pp. 173. (Constable: London, September 1976.) £3.95.

BORN into a matriarchal society in the wild bush of Africa, captured and transported to the Jardin des Plantes in Paris, the 4-year-old elephant was then exchanged for a rhinoceros, a piece of trading which almost certainly saved him from a stewy end in the seige of Paris of 1871. A more tranquil period followed with little to do to earn an almost limitless supply of buns other than carry wide-eyed children on rides round London Zoo.

Jumbo, for this was his name, thrived in London and grew apace. Jumbo was no more than a name of African origin but one which was to come into everyday use and be synony-

mous with largeness. The period of adolescence ended in pain of two kinds, the physical pain from abscesses which formed following the breaking of both tusks and the pain and puzzlement of puberty which seems to afflict the male elephant in the constraining environment of captivity.

The physical pain was assuaged by the surgeon's lance but the latter required very pragmatic action, if the embarrassment of an Elephant amok in the Zoo was to be avoided, and resulted in Jumbo's sale to Mr Barnum, the great American showman. The bill of sale was easy; the delivery of a reluctant elephant was, however, fraught with difficulties, difficulties that resulted in enormous publicity which promoted the forthcoming arrival to the American people and increased the anguish of the British public at the loss of their animal hero.

How little times have changed. Good organisation and one suspects the right kind of word from the animal's keeper, who could not but fail to profit from the increasing fame of his charge, and despite attempts for an injunction against the sale, Jumbo was loaded on the *Assyrian Monarch* and after an uneventful journey arrived in New York in April 1882. There then followed the razmataz of the American circus life which continued until Jumbo's death following a collision which Special Freight Train number 151 just over 3 years after his arrival in the New World.

The author draws from many sources to relate the tale of Jumbo's life and times. He links together the extracts from that period in a way which at times I found rather tedious, phrases like "yester year's politicians prize from China" to describe Mr Ted Heath's contribution of Pandas to London Zoo is but one example. But this could not spoil a fascinating story of a most unusual animal. The book is a must for all Jumbophiles.

R. J. Wheeler

Mr Wheeler is Director of the Royal Zoological Society of Scotland.

The Bethnal Green Museum of Childhood, London, UK, has mounted a special exhibition (November 25, 1976-January 23, 1977) of material related to the story of Jumbo. The exhibition also includes a wide variety of examples of the elephant in art, and contains toy elephants and a selection of photographs and drawings from children's books.



Jumbo with admirers at London Zoo

Photo: Zoological Society of London

Snapshots in the field

Field Photography: Beginning and Advanced Techniques. By Alfred A. Blaker. Pp. xxi+451. (Freeman: San Francisco and Reading, 1976.) \$19.95; £12.60.

THE camera is used by scientists to observe, collect and record data and to help communicate the results to fellow workers. Most government scientists can call on the services of professional photographers who produce photographs that are technically superb. But even these fortunate scientists, as well as ones without such facilities available, need to take photographs themselves as the opportunity presents itself, and this is especially true for those whose work takes them into the field.

The scientist who takes his own photographs has the advantage that he knows exactly what he wishes to portray. The result is, however, too often spoilt and rendered unfit for publication by lack of the proper equipment for the job, or the lack of rudimentary technical knowledge. One of the primary aims of *Field Photography* is to provide sufficient information for the scientist to overcome these difficulties. All too often articles and books on modern photography are

either technically too complex for this purpose, or consist merely of a series of photographs joined together with the minimum of words or explanation. *Field Photography* is noteworthy for its balance of text and illustrations. It can be readily understood by the beginner and yet contains much that is of interest and help to the photographer with some knowledge or one who has experience in some other branch of photography.

The steps needed to perform a certain operation are always given clearly and at length. Sufficient information is always conveyed for the reader to understand the reason for the processes involved, but the theory underlying the operation is usually only given at length when needed to enhance the practical performance. And the author does not dwell unnecessarily on the chemical or physical aspects of photography.

The book is divided into three major parts. In the first, the reader is skillfully piloted through the maze of photographic equipment and materials that are available, including the basic types of cameras, lenses, extension tubes, bellows, filters, light meters, flash meters, tripods, film and printing paper. The particular advantages and disadvantages of various items of equipment are clearly discussed: even veteran photographers should occasionally pause to reflect

whether the system they have adopted or evolved is really the most suitable for the job at hand, or whether it merely reflects ingrained habit.

In the second part of the book general photographic techniques are reviewed, emphasis being placed on: picture composition; exposure, including the use of light meters and electronic flash techniques; the purpose and use of filters, especially as they relate to scientific recording in the field and their use in infrared and ultraviolet photography; and darkroom procedures.

The third part is specially concerned with field photography techniques and is in many ways the most valuable. There can be few photographers who could not learn something from it. The subjects discussed include: climatic problems and protection of equipment; high resolution techniques, including camera handling, film processing and printing; macro-photography, including the use of supplementary lenses, teleconverters, extension tubes, and lens reversal; use of telephoto and wide angle lenses; techniques of stereophotography; and use of fill-in flash. The few pages in this section that are devoted to the correct disposal of photographic waste material and the avoidance of damage to the environment would seem unnecessary to the scientist who habitually works in the field.

The book is well produced and the clarity of presentation is enhanced by some excellent and purposeful black-and-white and colour illustrations, and many line drawings, figures and tables. Included with it is a booklet in which many of the mathematical formulae and tables used to calculate lens apertures and shutter speeds are reproduced, so the photographer can have readily available information with him in the field.

Field Photography is good value, especially if it succeeds in its object of instructing the scientist who buys it to take photographs outdoors efficiently and economically.

John B. Free

John Free is an entomologist at Rothamsted Experimental Station, Harpenden, UK, and a professional natural history photographer.

Volcanoes

Volcanoes. By Peter Francis. Pp. 368. (Penguin: Harmondsworth, Middlesex, July 1976.) £1.50. *Volcanoes*. By A. and L. Rittmann. Pp. 128. (Orbis: London, 1976). £4.95.

"THERE are many books on the market called *Volcanoes* . . . so why write yet another?" Thus begins Peter Francis in his addition to the *Pelican Original* series. And he has his own answers to the problem. Volcanology, he explains, is a fast-moving discipline, both conceptually and spatially. Studies that once encompassed little more than explicit phenomena on the surface of the globe now extend from the Earth's core to the far side of Mercury. Then there are the hidden implications of volcanoes. On the surface a volcano may be just a volcano, but an eruption has far reaching ramifications often ignored by other authors. And finally, most treatises on volcanoes are textbooks.

Peter Francis wanted to write one free of "academic pretensions" for a less than specialist readership, and his attempt at meeting that objective is an unqualified success. Here is a gem of a book; from the outset his knowledgeable fascination with volcanoes and all they represent shines through. Gifted with a lucid and breezy style he carries his narrative across every major facet of volcanology, penetrating surfaces just deeply enough to provide sufficient foothold for the next conceptual step. Nobody will get lost along the way, and few will want to abandon the journey.

The book proceeds logically, condensing a mass of detail as it does so. It starts clearly with a description of

volcanic distribution—the Mid-Atlantic Ridge, the Pacific Ring of Fire, and so on. This, of course, is merely plate tectonics from another angle, but it provides the author with the opportunity of introducing the fundamental geophysics of volcanology and all that that entails—from basic mantle chemistry and convection cells, through oceanic magnetic anomalies, to the Low Velocity Zone.

The author proceeds to paint a vivid picture of what volcanoes are and what they do. The scene is set with a straightforward descriptive chapter on three diverse "classic eruptions"—Vesuvius in AD 79, Krakatoa in 1883, and Mont Pelée in 1902—which, with the graphic inclusion of asides on the plight of unfortunate victims, provides a clear idea of the varied ways in which volcanic action can manifest itself.

But it is the following chapter which perhaps best highlights the author's skill as a scientific narrator. Dealing with the classification of eruptions, again everything is there—central and fissure eruptions, basaltic flood eruptions, Vulcanian and Strombolian eruptions, Hawaiian eruptions, and so on. The obvious pitfalls are confusion and complexity; but Francis does not trip up, making his central point clearly and concisely.

Lava types (a few words here on mineral chemistry and petrology), pyroclastic falls and flows, volcanic morphology and volcanic evolution are then considered, and by the end of chapter 7 the story could have been complete on many counts. But Francis is scarcely more than half way through. As he says bluntly: "a volcanic eruption does not take place in an environmental vacuum". So we get *hlaups* and *jökullhlaups*, volcanic pollution, *tsunamis*, climatic effects, volcanoes and the origin of life, and even vol-

canoes as money spinners—ores, geothermal power, and the like. A chapter on volcanic prediction and mitigation (La Soufrière is topical), a journey out to Mercury (by way of the Moon and its history, and Mars), and at last the text reaches its breathless end.

Peter Francis' *Volcanoes*—361 pages crammed with information—is thoroughly comprehensive and crystal clear. Written to inform non-specialists, it does so with panache.

By comparison, *Volcanoes* by Alfred and Loredana Rittman is a strange offering, the more so in view of the senior author's standing in his field. As the dust cover proclaims, he is indeed a "world authority"—an ex-President of the International Association of Volcanology, and founder of the International Institute for Research into Volcanology.

With that in mind his latest contribution emerges as a sadly disappointing work. It really belongs to that peculiar breed known as the 'coffee-table' book. Without doubt, the 120-odd full-colour pictures are breathtaking—of the type that beg to be looked at time after time. But the real difference between this book and Francis' (and given the similarity of textual approach the comparison is valid), is that whereas Francis writes to invest his photographs with life, the Rittmanns' present a relatively abtuse and somewhat superfluous text that is far outdazzled by the pictures. So if you have a coffee table and a penchant for Kodachrome catastrophe, the Rittmanns' *Volcanoes* is the book for you; if not, spend your money on the other option.

Allan Piper

Allan Piper is Assistant Editor of The Oilman.

Astronomy for the amateur

STARS and planets, galaxies and space are topics of tremendous interest to many non-scientific members of the general public. In fact astronomy is second only to natural history in the layman's top ten of scientific subjects, and this is amply reflected in the plethora of popular astronomy books published every year. These books are difficult to classify but the seven under review fall into four basic groups, the encyclopaedic all-you-want-to-know about astronomy from A to Z; the picturesque, where a large collection of glossy photographs are interspersed

by brief descriptive texts; the armchair tomes for the fireside astronomer; and the 'get-up-and-go' books which encourage you to actually go outside and gaze, hopefully with wonder, at the heavens.

First let us consider the two encyclopaedias. These ABCs of astronomy are ideal for people who want to look up simple astronomical facts and who want quick answers to questions without having to study a lengthy chapter in a book. A. Weigert and H. Zimmermann's *Concise Encyclopaedia of Astronomy* (Adam Hilger: Bristol, £9) is a revised and expanded second edition of the original *ABC der Astronomie* first published in 1967. There are over 500 pages of facts, many illustrations, sixteen plates and a fine collection of star maps going down to

fifth magnitude. This book is for the advanced amateur and is also an excellent reference book for first-year university students. Simple mathematical relationships between quantities are effectively used and the tabulation of stellar and planetary data is first rate.

The Illustrated Encyclopaedia of Astronomy and Space (Macmillan: London, £7.95), edited by Ian Ridpath, contains contributions from twelve or more people from diverse astronomical backgrounds. This encyclopaedia tries to bring together astronomy and space science, and amongst the 1,000 entries are some very informative tables giving details of such space programs as Apollo, Surveyor, Soyuz, Discoverer, Salyut and Skylab. The book is equally divided between space and astronomy—for example, the planet Saturn is allocated as many column inches as the Saturn rocket. In fact I think the book goes too far into space—Frank Borman the Apollo 8 mission commander is allocated eleven lines, and the fundamental astronomical concept of black-body radiation is afforded scant reference in the section on spectroscopy. It is well illustrated but is much more simplistic than the work by Weigert and Zimmermann. Ridpath's book reads more like an astronomical *Guinness Book of Records* and seems to be aimed at people in their early teens.

The Southern Universe (Macmillan: London, Melbourne and Sidney, £4.95) by Lennard Bickel is so lavishly illustrated that it can almost take its place with those other 'coffee-table' books designed just to be flipped through to look at the pictures. This is, however, a bit unfair as the text is worth reading especially if you can overcome your dislike of journalistic flamboyance:

"The Clouds of Magellan are portals to outer space. Their location—50,000 parsecs above the South Pole—is our threshold to the greater universe, an arena so vast it is better described as—the cosmos. This is the great empyrean of the heavens, the region of supreme creation where scientific astronomy is joined by cosmology and modern physics in the search for understanding of the true Beginning."

Yeuk! There again it might be just the thing for the kiddies. Interestingly the book is written by an Australian, with the cooperation of many Australian astronomers and with a strong emphasis on the southern sky, a refreshing change from the usual bias towards the northern hemisphere.

Isaac Asimov writes for the armchair astronomer, and his book, *Eyes on the Universe: A History of the Telescope* (Andre Deutsch: London, £4.95), sets out to tell two stories. First, a fascinating account of the technological

achievement that developed the telescope from the first Heath-Robinson contrivances of the pioneer astronomers to the giant optical and radio telescopes of today. And second a narrative of mankind's supreme intellectual adventure as he reaches out and tries to comprehend the nature, scope and origin of the Universe. The book succeeds admirably and tells its rather complex tale, without the use of mathematics, in a clear and succinct way. A perfect example of good scientific popularisation.

Astronomy for the Amateur (Macmillan: London, £2.95) is a short, well illustrated introduction to astronomy. John Gribbin has written for those people "who have noticed the fascinating lights in the sky and would like to know a little more about them". It is a guide for people embarking on astronomy as a hobby, tries hard to keep alight the fascination and wonder of this exciting science, and succeeds very well.

Moving outside we come to James Muirden's *Astronomy with Binoculars* (Faber and Faber: London, £4.95 cloth, £2.75 papercover), a new and revised edition of the book first published in 1963. Astronomy is an intimidating science, many newcomers imagining that astronomical research can only be carried out in big observatories. Muirden tells us all to get outside on the frosty winter nights and train our binoculars on the sky. There are chapters on the observation of the Sun (needless to say *not* with binoculars), Moon, planets, comets, meteors aurorae and stars. In each case the author describes in detail what we should be looking for and prefaces these descriptions by a brief introduc-

tion to the physical conditions prevailing on the object. This book conveys all the excitement and challenge of astronomy, and the owner of a pair of binoculars will find the book worth every penny of its cost.

Finally, I come to *Stars and Space 77* (Independent Newspapers: London, £1) a ninety-six page, magazine-style paperback, which firstly describes the night sky for every month of the coming year—giving well drawn and very clear star maps plus details of planetary positions and lists of interesting stars to look out for. The second half is given over to reviews of the major advances and discoveries during the past year. These three- to four-page articles cover such subjects as exploding black holes, X-ray satellites, other planetary systems, the solar influence on the Earth's weather, comet West, and Viking. The author list reads like a Who's Who in Astronomy, and Ian Ridpath, again editor, must be congratulated on an excellent publication—fantastic value for money at only £1. Heaven help the sales of Patrick Moore's *Yearbook of Astronomy*.

Looking at all these books the question to ask is: "Which, assuming you are a young astronomer or a recent addition to the realms of amateur astronomers, would you like to find in your stocking on Christmas Day". The choice is enormous, but for me the answer is simple. James Muirden's *Astronomy with Binoculars*, wins hands down, with Ridpath's *Stars and Space 77* also making a superb and cheap stocking filler.

David W. Hughes

David W. Hughes is a Lecturer in the Department of Physics at the University of Sheffield, UK.

Ecology of human skin

The Life That Lives on Man. By Michael Andrews. Pp. 183. (Faber and Faber: London, 1976.) £4.95.

TELEVISION has brought many aspects of science vividly to huge audiences, and the programme *The Life That Lives on Man* did this most successfully. It gave an accurate picture of the human skin, and had shown—with shots of dust pouring out of our underclothes—how we shed some ten thousand million tiny skin scales a day, losing in a year a weight of over a pound from our tissues. It had also shown photomicrographs of the bacteria and the various arthropods which live on and in the skin, and described the diseases some parasites may cause. There were accounts of the research

on these parasites, and reconstructions of some of the unusual experiments (including those in which man was the victim) which contributed to this research. It was obvious to the viewer that an immense amount of work had gone into preparing the script, and in taking and editing all the photographic material. It seemed a pity that so much effort should produce so little permanent result, for even the most attentive viewer retained only a fraction of the information which illuminated his screen when the programme was presented on television.

It was therefore an excellent idea when Michael Andrews decided to write "the book of the film". In it he has been able to include much that his research revealed which could not be shown on television because of pressure on time or because not all aspects of the subject are equally photogenic. Mr Andrews has produced a fascinating

Catching lice, by Bartolommeo Pinelli (Rome, 1781-1835)



Photo: Mansell Collection

book, scientifically accurate and yet free from irritating jargon. It should appeal to the non-scientist, and yet has something to teach the specialist, including the dermatologist and the parasitologist.

Although the text is enlivened with historical anecdotes and literary allusions, with poems about the parasites and epigrams at the expense of the parasitised, the book is fundamentally an exercise in ecology—the ecology of the human skin. We learn that the millions of bacteria which inhabit it are normally in balance, although many are potentially the cause of serious disease. When man does not interfere by washing too frequently, or applying potent chemicals to neutralise natural body odours (not BO, which is generally a pathological symptom) and to prevent natural sweating, the various micro-organisms can usually co-exist in mutual harmony. Dangerous pathogens are somehow prevented from increasing to damaging numbers. It is clear that many bacteria and other micro-organisms are beneficial and not harmful to their hosts, a situation recently described in Bernard Dixon's book *Invisible Allies*. The skin is seen to behave much like other, larger, ecosystems.

Stability may, however, be upset in all ecosystems, and the skin is no exception. Dangerous bacteria do sometimes get out of control, perhaps because man, with his unguents and cosmetics, has upset the balance. It must also be admitted that it is difficult

to determine in what ways most arthropod parasites contribute to the well-being of the host which supports them. Lice suck our blood, irritate our skin so that we scratch and admit pathogenic bacteria to make the wounds fester; and the insects also infect us with dangerous rickettsiae which cause epidemic typhus. Fleas bite and inoculate us with the plague bacillus—the cause of the deadly "Black Death" of the Middle Ages. These infestations

Coffee-table medicine

Triumphs of Medicine. By H. Keen and J. Jarrett. Pp.193. (Paul Elek: London and New Hampshire, 1976). £12.50.

LAVISHLY illustrated books on art, history, architecture and travel have proved so successful that from time to time a publisher attempts to apply the same technique to a scientific theme. The size (30×23 cm) of this Paul Elek production puts it firmly into the 'coffee-table' class, but it is far from a typical example—except in its high price.

Professor Harry Keen and Dr John Jarrett set out to describe the main achievements of medical science as seen against a historical background. Their 17 expert contributors have served them well in identifying the important advances made in the control of infections, in anaesthesia, and in surgery and many new techniques of

seem to be singularly one-sided affairs. The reason may be that the arthropods are, for the most part, recent additions to the fauna of the human skin. The germs they carry may be better adapted to mammals other than man. Both vectors and micro-organisms may not yet have had time to develop anything like a symbiotic relationship with man.

There is in fact some suggestion that such relationships may be evolving. Although the arthropod parasites may not benefit man they do seem, in the majority of cases, after an initial rather heavy infestation, to decrease in numbers to produce a balanced, rather small population which does not cause intolerable discomfort. Thus the majority of lousy children, including those with long-term chronic infestations, have only about a dozen lice on their heads. Most patients with chronic scabies support only a similar number of *Sarcoptes* mites. Perhaps the idea, prevalent among Victorian slum dwellers, that a few lice on a child's head was a sign of health may have some foundation. Perhaps low and relatively harmless populations of ectoparasites prevent other and more dangerous invaders from damaging our skin. The ecological ideas put forward by Michael Andrews in this excellent book may stimulate scientists to investigate these problems anew.

Kenneth Mellanby

Although Professor Mellanby was at one time a Reader in Medical Entomology, he has become better known for his work at the Monks Wood Experimental Station, Huntingdon, UK, and for his success in conveying the complexities of science to a wider audience.

diagnosis and treatment. They have also explained the growth in our understanding of the structure and biochemistry of the body in health and disease. Although some chapters, such as Professor Roy Calne's account of the kidney and of organ transplantation are models of clarity, others—though technically competent—are fogged by patches of the impenetrable jargon characteristic of so much medical writing. Perhaps because the editors realised that too many of the contributions were too detailed for a non-specialist readership they included a glossary of technical terms; but even that assumes a considerable scientific vocabulary.

Books of this kind stand or fall on their appeal to the eye. A high standard has been set by designers such as George Rainbird (responsible for successes such as Douglas Botting's biography *Humboldt and the Cosmos*), whose formula depends on the faultless reproduction of large numbers of plates of exceptional beauty, many of

them in colour. In contrast, the reader browsing through *Triumphs of Medicine* finds no colour; and though every page has a picture and many are full plates, few are really eye-catching. Many of the full-page illustrations are taken from X-ray films; again, their technical quality is excellent, but few non-medical readers will find them informative. Virtuoso examples of the radiologist's skills, such as a film showing exactly where blood is leaking from an artery into the intestine, mean little to someone who has never seen an angiogram before. The same criticism may be made of the photomicrographs; without explanation they are meaningless to the non-medical eye and they have little visual impact.

There is an exciting story to be told in the growth of medical expertise as part of the twentieth century explosion of scientific knowledge, but the telling must be adapted to the audience. Here the target is far from clear, for a collection of intellectually demanding essays has been confused and interrupted by a haphazard mixture of technical illustrations, historic cartoons, line drawings, portraits, and journalistic photographs. The packaging is not consistent with the content, and in consequence the result will please neither the specialist reader nor the curious layman.

Tony Smith

Tony Smith is Assistant Editor of the British Medical Journal and Medical Correspondent of The Times.

Islamic masterwork

Islam and the Arab World. Edited by Bernard Lewis. Pp. 360. (Knopf in association with American Heritage: New York, 1976.) \$35.00. (UK edition (*The World of Islam*) published by Thames and Hudson: London, 1976. £12.50.)

THE publication of this first American edition of *Islam and the Arab World* is truly an occasion for celebration.

MAGNUS PYKE BUTTER SIDE UP!

or The Delights of Science

Does bread and butter fall more often butter side down or up? Did Popeye really owe his vigour to can after can of spinach? Ask Magnus Pyke an apparently silly question and he will give an eminently sensible answer that reveals the inner workings of science. Following up many of the trails started during ITV's very popular series *Don't Ask Me* and heading off on many new ones Dr Pyke has gathered a rich crop of scientific eccentricities.

Drawings by ffolkes
8 pages of photographs
£3.95

**JOHN
MURRAY**

Furthermore, the celebrants need not be Arabic or Islamic scholars alone, for the volume is sure to be appreciated by the complete spectrum of professionals, from artists to zoologists. At a casual glance, this profusely and beautifully illustrated book might appear to be a classier version of a typical multi-colour 'coffee-table' book, which are more often valued for their role as icebreakers in a conversation, rather than for their intrinsic value. That would indeed be an incorrect impression because in the (effectively) fifteen chapters of the book, the reader is gently but carefully guided through topics such as the explosion of Islam in the Arabian peninsula and beyond, the art and architecture of the vast Dār al-Islām, ranging from Andalusia to Bengal, its literature, natural sciences, music and a bird's-eye view of its up-to-date political history. Each chapter is written by unquestionable authorities on their subjects, seemingly handpicked by Professor Bernard Lewis not only for their scholarship but also for their ability to communicate with the lay public and, what is more important, for their unusual sense of balance. In fact, there are perhaps only one or two chapters which occasionally show lapses in this latter mentioned quality.

The title of the book could be slightly misleading in that a potential reader may not realise the existence of whole chapters in the book dealing with Muslim Spain, Iran from the Safavids onwards, the Ottoman empire, and last but not least, Muslim India. Thus, in the strict definition of the Arabist, the present volume deals with the whole of Islamic culture (or "Islamicate" culture as the late Marshall Hodgson would have said) and not just Arabic civilisation. The corollary of that is the book's involvement with the whole of Dār al-Islām since the Prophet's birth.



Musician with oblique flute, Persian album painting, 1560-70

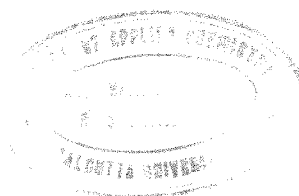
Courtesy Sotheby's, London, UK

The chapter entitled "The Scientific Enterprise" deserves special notice because of the sparkling characteristics of this long essay. It is well-known to students of Islamic science that other than the articles in *Encyclopedia of Islam* (incomplete second edition) or the *Legacy of Islam* (1974), there is not even a satisfactory monograph on the subject of Islamic science. Obviously, Professor Sabra's article in twelve pages (even if they are quarto-size pages) does not remove this inadequacy, but it goes a long way towards initiating an intelligent and non-partisan discussion about the paradox of the coexistence of rationalists (falāsifa) and the orthodox religious thinkers (Ahl as-Sunnah).

Although the book is a masterwork, I would like to slip in a few minor criticisms here: the dust cover is too garish and projects too narrowly martial an image for such a world culture; the excellent chapter on music does not mention a word about Hindustani music; and for a confirmed epicure it is distressing to find not only a lack of mention of Turkish cuisine which vies with the French and Chinese as a primary world cuisine but that even the word 'food' is never mentioned. Perhaps the coffee-table culturists would at least have liked to know the origin of the word 'coffee'!

Subir K. Banerjee

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A few months' relief, but what then?

So, at the eleventh hour, a way has been found for the Science Research Council (SRC) to pay its international subscriptions, notably to CERN, without thereby bankrupting all its other activities. It isn't clear as yet that the hat which had embarrassingly to be passed round all manner of scientific establishments for donations to the cause will be passed back intact, but there is hope that the modest sum that was found therein will eventually get back to the kindly oceanographers, invertebrate virologists and so on who did their bit. But at the risk of boring our readers even more—and we promise to say nothing further on the subject in 1976—there are still some unanswered questions about the future of international subscriptions in times of inflation and a sliding exchange rate.

The somewhat disturbing truth about the resolution of the problem for this financial year (to March 31, 1977) is that it has been possible only by some dexterity in shuffling funds and reinterpreting rule books at the Department of Education and Science (DES) and the Treasury. There had always been a feeling around that the cash-limits problem could be circumvented somehow, and so it turned out. Some money (about £2.5 million) which had been earmarked for university purposes in the form of an operational reserve was early on seen to be spare and the Treasury had pointed this sum at the SRC. But the real breakthrough came when some very careful reading of the rulebooks, and, one suspects, some very slight reinterpretation of them, showed that a sum of £1.6 million previously thought to be the responsibility of the research councils was found to be chargeable to other departments involved in commissioning research in council establishments. Not £1.6 million was saved that way, but double it!

If this means a somewhat relaxed Christmas for all concerned, it doesn't necessarily mean good news for the

financial year 1977–78. In the next few weeks the results of Britain's widely leaked commitment to the International Monetary Fund to reduce public spending in exchange for a large loan are bound to trickle through the Whitehall machine. It would be foolish to think that in some way science can be buffered from these cuts, either in the sectoral departments with a science and technology constituent or through the Department of Education and Science. All of which means that there are not only bound to be several uneasy months as decisions are made on whether staff levels are to be maintained or whether the cuts are to be passed on to sub-contractors, university departments and the like; it also means that the monolithic international subscriptions, not open to little bits of trimming here and there, are bound to become more exposed, occupying, as they will, a growing fraction of SRC's budget. And the idea of strict cash limits, which has been at the root of this year's difficulties, is likely to be around for years to come judging by the general enthusiasm in Whitehall for them.

Thus, although this year's patching-up operation is welcome indeed, the lack of any sort of systematic resolution of the problems brought on by variable exchange rates will leave us with a perennial headache, unless the pound starts to move the other way. The waste of time, the stirring up of animosities, the internal conflict that followed this year's difficulties are not things that ought to become an annual feature of the life of practitioners of big science. It still seems to us most desirable that within the near future a mechanism will be found by which international subscriptions are not jeopardised by currency fluctuations. Other countries manage it—surely if DES contains people clever enough to convert to a £1.6 million bill into a £1.6 million IOU, it contains people clever enough to devise means of saving themselves the bother in future years. □

The breeder reactor: a fossil fuel viewpoint

David Merrick considers what economic and technical factors would make the fast breeder reactor a viable option

THE concept of the fast breeder reactor is elegant and simple: to generate electricity and, at the same time, to produce additional fuel from the uranium discarded by the existing thermal reactor system. Without the breeder reactor, it seems likely that the role of nuclear energy will begin to be constrained by the price and availability of uranium at about the turn of the century. There is, however, no consensus on the desirable, or even possible, contribution of breeder reactors to future energy supplies. Foremost among the uncertainties discussed in the current debate are the questions of safety and environmental impact. Assuming concern in these areas can be satisfactorily and economically resolved, there still remain questions on the technical and economic limitations of the use of breeder reactors.

Performance of the breeder system

In making the case for the breeder reactor, the most commonly quoted index of performance is that 30% to 60% of the energy available in the uranium fuel can be liberated, compared with about 1% in most thermal reactors. The exact value of the efficiency depends on the plutonium losses in reprocessing, the 60% figure being valid only if these can be limited to 2%.

In addition to approximately 4 tonnes of plutonium in the core, a 1 MW breeder reactor contains about 50 tonnes of depleted uranium in the core and blanket. This is converted by the reactor to plutonium (and other elements) and typically, a further 50 tonnes of depleted uranium are required as replacement during a lifetime's operation. A single breeder reactor will, therefore, utilise only 15% to 30% of the uranium required for its operation, if the initial charge is included.

The uranium inventory can be considered as a form of storage and remains useful provided there is a continuing programme of breeder reactor construction. If, however, an expanding programme of breeder capacity is planned, a further constraint, the availability of fissile material for the reactor core, is encountered. Although it would be technically possible to use highly

enriched uranium for this purpose, to do so would be costly, and the reactor performance, although improving slowly during the lifetime of the plant, would not initially be in the 'breeding' regime¹. For these reasons the design of breeder reactors, and scenarios for their introduction (for example the United Kingdom Atomic Energy Authority's 1974-75 evidence to the Royal Commission on Environmental Pollution²), concentrate on the use of plutonium as the fissile material.

It is expected that the Liquid Metal Fast Breeder Reactor (LMFBR) will breed 20%-25% more plutonium than is required as fuel. This excess plutonium can be used as inventory for further breeder reactors. A crucial parameter affecting the strategy for the introduction of breeder reactors is the doubling time, that is, the time taken for a breeder reactor to produce sufficient plutonium to provide the initial fuel charge for another similar reactor. The doubling time depends not only on the breeding gain of the reactor itself, but also on the delay time and losses in reprocessing the partially burned fuel. At the present state of reactor and reprocessing technology, the doubling time would be more than 50 years³, although this is expected to be cut to a period in the range 25 to 40 years for commercial reactor systems (at a 70% load factor). As neither the reactor nor the reprocessing plant are likely to be demonstrated on a commercial scale for several years, it seems prudent to assume a mid-range value of, say, 33 years for planning purposes.

It is claimed that further reductions in the doubling time could be obtained by using carbide instead of oxide fuel, or a gas-cooled reactor instead of the present liquid metal designs. However, even the achievement of a 33-year doubling time in an acceptable commercial system will entail a substantial development programme, and whether sufficient incentive would then remain to embark on further such programmes is not clear.

At the beginning of a programme of breeder reactor construction, the plutonium required for the reactor core would be obtained as a by-product of the thermal reactor programme. In most situations, the available stocks of plutonium would be rapidly exhausted, and the rate of construction of breeder

reactors would then be limited by the rate at which plutonium becomes available from existing thermal and breeder reactors. In the UK, for example, stocks of separated plutonium would, at present, enable 1 GW of breeder capacity to be built⁴. This should rise to about 7 GW in 1985.

Whether the breeder programme can ever become independent of the thermal reactor programme depends on the growth rate of the nuclear electricity system, and the doubling time. At a doubling time of 33 years, plutonium production from the breeder reactor system can only sustain a growth rate of around 3% per annum. For the growth rate of the nuclear electricity system to be greater than 3% per annum, it is necessary to construct both thermal and breeder reactors.

In this situation, the efficiency with which uranium is utilised by the overall system of breeder and supporting thermal reactors is considerably lower than the value of 60% claimed for the breeder reactor alone. This is illustrated in Fig. 1, which shows the uranium utilisation for the system of breeder (LMFBR) and supporting thermal reactors (PWR) at various growth rates. Also shown, for comparison, is the uranium utilisation if the CANDU thermal reactor, with plutonium recycle, were to be used. This system performs better than the LMFBR/PWR system at growth rates above about 5% per annum.

If, therefore, a rapid build-up of nuclear capacity were desirable, the LMFBR/PWR system would, during the period of growth, offer a relatively small improvement in uranium utilisation over the PWR, and little or no improvement over the CANDU system. Only if the projected plutonium doubling time of commercial breeder reactor systems were to be substantially reduced could a high growth rate in breeder capacity be achieved, but there is no evidence at present that this would be technically or economically possible.

Growth of the breeder system

Besides limiting the overall efficiency of uranium utilisation in the nuclear reactor system, the availability of plutonium for the cores of breeder reactors

Table 1 Estimated uranium import requirements 2000-2020 AD

Growth rate (% per annum)	LWR + LMFBR	LWR +Pu recycle	CANDU +Pu recycle
1	39,000	105,000	51,000
3	76,000	214,000	103,000
5	220,000	433,000	208,000

also acts as a constraint on the rate at which breeder capacity can be introduced. This can be illustrated by considering an idealised situation in which the commissioning of new power stations precisely matches the steadily growing level of demand. The maximum rate of introduction of breeder reactors is shown in Fig. 2 if, at year zero, new power station construction is suddenly switched from fossil fuel to nuclear.

At annual growth rates in electricity demand below 3%, breeder reactors can eventually account for all nuclear power generation, although to achieve this takes at least 50 years. At higher growth rates, the ultimate contribution of breeders is limited, as discussed above.

For a given electrical output thermal reactors generally produce plutonium at least as quickly as breeders. If the introduction of breeders is delayed, plutonium stocks can, therefore, be built up, enabling breeder capacity to increase rapidly once the breeder programme begins. Even a delay of 30 years does not significantly affect the time required for the breeder to reach the maximum possible contribution, as shown in Fig. 2.

Forecasts of the rate of growth of breeder reactor capacity and the impact of uranium imports to the UK are complicated by the present over-capacity of power stations and the plutonium stocks from existing stations. Approximate estimates of uranium requirements in the first 20 years of next century are given in Table 1, assuming that an early decision to go ahead with a demonstration-scale breeder reactor is made. This would enable the first orders for commercial plants to be made by the middle/late 1980s for commissioning in 1995.

The introduction of breeder reactors will, on this timescale, only reduce uranium consumption to just under half of the requirements of a nuclear programme based on the PWR alone and will show little advantage over the CANDU thermal reactor. The choice of thermal reactor system can, therefore, have a similar impact on uranium requirements to the breeder on this timescale, and cannot be divorced from the decision to pursue breeder technology. The sixty-fold improvement in uranium utilisation frequency claimed for the breeder will not be realised for the system as a whole until well into next century. Although the medium-term savings will be worthwhile provided uranium prices are high, breeder technology cannot ensure a secure, indigenous supply of energy, effectively insulating the UK from the expense of high cost uranium, for more than half a century.

Economics of breeder reactor systems

Although it has been claimed that the capital cost of fast breeder power stations need not be greater than that of thermal stations, it now seems likely that, even if there are no major technical difficulties, the cost will be at least 25% more than that of a PWR. In order to be economically viable, this additional capital investment must be paid for by the lower fuel costs. At present, the cost of uranium ore accounts for less than 10% of the total generation cost so that, other factors being equal, a substantial increase in the price of uranium would be necessary to justify the extra capital cost of a breeder reactor.

This is confirmed by a more detailed analysis, which shows that the approximate cost of uranium ore needed for a breeder to produce electricity as cheaply as a thermal reactor is directly proportional to the additional capital cost of the breeder. (The plutonium produced by the breeder and thermal reactors is credited with its value as a supplementary fuel in thermal reactors, although in the case of the breeder this credit is more than offset—at a 10% discount rate—by the cost of the initial charge of plutonium.) Although this analysis is necessarily approximate, such a relationship means that the FBR is unlikely to give visible economic benefits until uranium prices have increased to \$100–\$200 per pound of U_3O_8 . This represents an increase in real terms of three to six times.

The rate at which uranium prices will move upwards depends on the future growth of world nuclear capacity and reserves and discoveries of high grade uranium ores. Neither of these can be estimated with any certainty and no consensus of opinion exists on the earliest date at which this consideration would be decisive in favour of the breeder. The UKAEA anticipates that uranium prices will increase to \$100–\$200 a pound when all the high grade ores are committed to existing nuclear programmes in the 1990s, and conclude that breeder capacity should be built up as quickly as possible. The Central Electricity Generating Board (CEGB), however, takes the view that the main justification for the FBR is in reducing requirements for uranium in the long term, as resources may be limited, rather than on economic grounds.

In order for nuclear energy to remain competitive for base load power generation duty at uranium prices in the range \$100 to \$200 a pound, fossil fuel prices would have to rise by between 50% and 100% relative to power station construction costs. Although, in the UK, power station coal now costs about 2½ times as much as it did three years ago (an increase even allowing

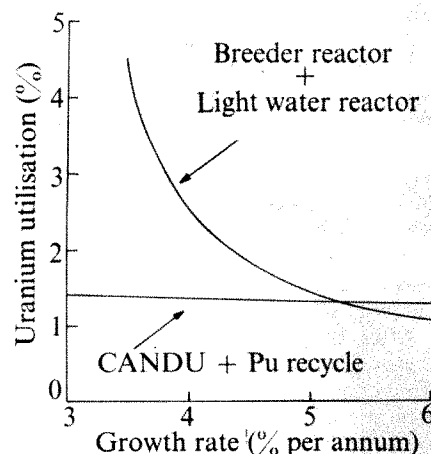


Fig. 1 Uranium utilisation in a steadily expanding system.

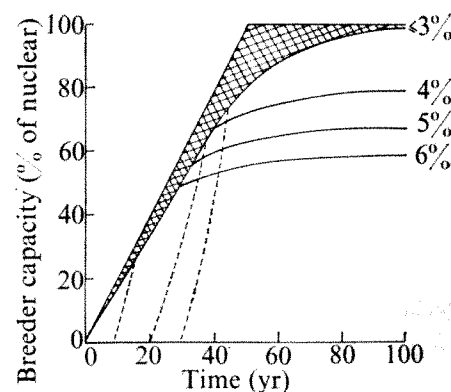


Fig. 2 Rate of growth of breeder capacity in an idealised system assuming various annual growth rates for electricity demand. For the 3% annual growth curve, the effects of delaying introduction of the fast breeder by 10, 20 and 30 years are shown by dashed lines.

for inflation), power station construction costs have risen by a similar amount over the same period, leaving the relativities in power generation costs not greatly different.

There is clearly a possibility that the increased cost of nuclear power generation brought about by high uranium prices will either reduce the competitiveness of nuclear electricity compared with other forms of energy or, should the price of the alternatives also increase, inhibit economic growth and consequently energy demand. In either case, the assumption of a continued high rate of growth in nuclear capacity after high grade uranium ores are exhausted appears open to question.

Fossil fuels

The justification for the breeder is essentially long term. No clear economic advantage is likely until uranium prices have risen three to six times above their present level. Furthermore, the contribution of a breeder/LWR system to reducing uranium requirements is unlikely to exceed that of an

efficient thermal reactor system until well into the next century.

If demand continues to grow so that it becomes impracticable to rely on thermal reactors, fossil fuels, and renewable resources for power generation, the breeder reactor would have an important contribution to make. It is against this scenario that the development of breeder technology could be considered a valuable assurance of adequate electricity supplies.

After the turn of the century, it is expected that crude oil and natural gas production will begin to decline. This, because of the unique advantages of fossil fuels, will encourage substitution either directly or indirectly, by coal. For that to occur most efficiently, new technologies for the combustion of coal and its conversion into substitute hydrocarbon fuels are required.

The development of coal conversion processes and breeder technology have certain similarities. In both cases, the construction of demonstration scale plants will soon be required. The earliest times by which each technology need be applied in the UK are similar, although both could, if required, be introduced before they become economic in order to forestall sudden increases in energy prices. Once the technology has been demonstrated,

however, the rate of commercial exploitation of coal conversion processes is not limited as for the fast breeder reactor, and a significant impact on energy supplies should be possible in a comparatively short period of time. Furthermore, the UK is already in a strong position on coal technology and the export potential of coal conversion processes should be no less than that of breeder technology.

The size of the relative contributions of breeder technology and coal conversion processes depends on whether electricity will continue to penetrate the energy market as prices increase. It is probable that substitute fuels from coal will be most effective competitors for nuclear electricity in the heating market. The estimated capital cost per unit of energy output of, for example, a plant to convert coal to substitute natural gas is about one third that of a nuclear power station, and the thermal efficiency is approximately double. Furthermore, the low load factors associated with the heating demand do not affect the economics of coal conversion as much as electricity generation. The use of nuclear energy for other than electricity generation does not look attractive at present, and would require considerable technical problems to be solved.

In conclusion, breeder reactors are capable of making a substantial contribution to energy supplies by using the uranium rejected from the thermal reactor programme. The availability of plutonium is likely to limit the rate at which breeder reactors can be built for several decades. If a vigorous development programme enabled breeder reactors to be introduced commercially by the 1990s, even although they would probably not be economic by this date, they would be unable to insulate the UK effectively from the need for expensive uranium imports until well into next century. The need to develop and demonstrate breeder technology can therefore be compared with the development of other medium-term energy options. Coal conversion processes, in particular, will be required to play a vital role in this timescale and merit no less attention and priority than breeder technology. □

I thank Mr L. Grainger, National Coal Board Member for Science, for guidance and encouragement, and the NCB for help with the preparation of this article. The views expressed are, however, my own and not necessarily those of the NCB.

¹ Leslie, D. C., *Aspects of Energy Conversion* (edit. by Blair, I. M., Jones, B. D., and Van Horn, A. J.), (Pergamon, Oxford, 1976).

² Royal Commission on Environmental Pollution, *Sixth Report: Nuclear Power and the Environment* (HMSO, London, 1976).

³ Vaughan, R. D., and Farmer, A. A., *Proc. Instn Mech. Engrs*, 190, 30/76, 163.

⁴ CEBG Corporate Plan 1976 (CEGB, London, 1976).

Dolphin dissonance

Colin Norman reports from Washington on the threat that tuna fishing poses for dolphins

IN the past 15 years, between 5 and 6 million dolphins are believed to have drowned in the nets of tuna fishermen. The slaughter of these highly intelligent, friendly mammals has sparked one of the most bitter and complex conservation battles ever waged in the United States. A number of environmental groups, backed to some extent by the federal government, are fighting to protect the animals, while the tuna industry, according to its spokesmen, is fighting for its own survival. The battle is now focused on regulations, due to take effect in January, which conservationists say are vitally needed to protect the dolphin schools, but which the tuna industry claims will put the United States tuna fleet out of business.

The dolphin owes its plight to the fact that, for unexplained reasons, some species frequently swim with yellowfin tuna—a type of tuna which is in high demand and which fetches the highest prices. Because the air-breathing dolphins swim on the surface, they have for decades provided

tuna fishermen with a convenient guide to the location of schools of yellowfin. Until the late 1950s, tuna fishing was mostly done by hook and line, and the dolphins lost nothing by showing fishermen the way to yellowfin schools. But a technological innovation in the industry rapidly changed all that.

The innovation was the development of a massive net, called a purse seine, which tuna fishermen spread around dolphin schools and then draw in to land the yellowfin beneath the dolphins. It is a very effective way to catch tuna, but the problem is that dolphins frequently become entangled in the nets and, when they can't get to the surface to breathe, they drown. During the 1960s, an average of about 400,000 dolphins a year were dying in tuna nets; since they have no commercial value, they were thrown overboard.

Because the dolphin schools play a valuable role in leading tuna boats to yellowfin, it is in the fishermen's interest to try to preserve the animals by reducing the slaughter. They began to experiment with various manoeuvres

and changes to the nets in the 1960s, but the killing continued virtually unabated until 1972. At that point, Congress stepped in.

Public concern

Public concern over the killing of marine mammals had been aroused by the plight of many species of whales, which had been hunted virtually to extinction, and the needless slaughter of dolphins by tuna fishermen consequently became an emotionally charged issue in the early 1970s. Congress responded to the concern by passing the Marine Mammal Protection Act (MMPA) in 1972.

A compromise between the argument that the tuna industry would be wiped out by a total ban on the killing of dolphins, and the need to protect the animals, the act essentially gave the industry a two-year grace period. By October 1974, the act said, the killing of dolphins by purse seiners must be reduced to "insignificant levels approaching zero".

Congress had been led to believe that such a requirement could be met. During testimony before a House committee in 1971, Joe Medina, a tuna boat captain, reported that trials with a modified purse seine had reduced the kill of dolphin in a 'set' to insignificant amounts. "This new net has been a

salvation for us, and I think we have the problem licked", he said. Unfortunately, his optimism was unjustified.

According to a study by a panel of experts last July, the total number of dolphin killed by all tuna fishermen, including non-US operators, dropped from 348,000 in 1972 to 217,000 in 1973, and it dipped again to 120,000 in 1974. But it increased last year, reaching 181,000. Clearly, the requirements of the MMPA were not being met and the Commerce Department, which was supposed to be enforcing the Act, wasn't doing much about it.

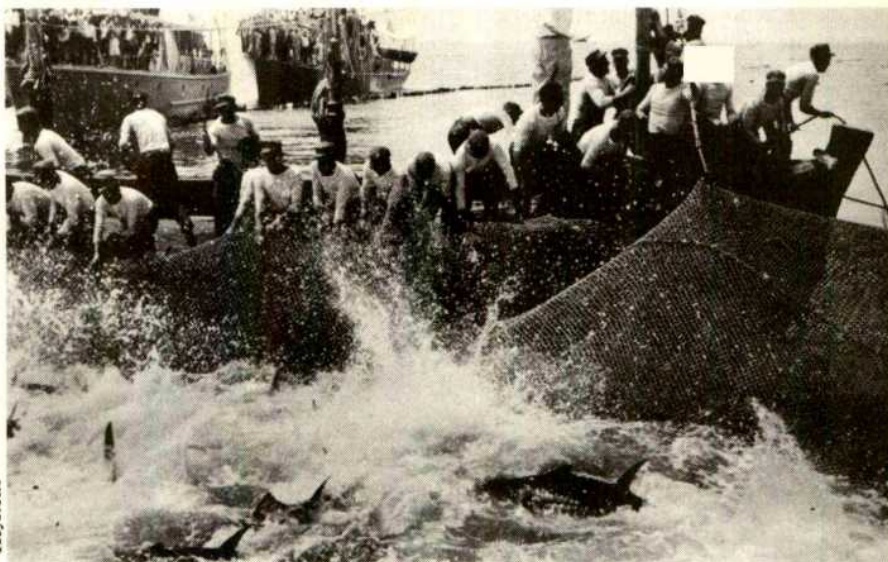
In December 1975, the Commerce Department moved. It announced that it would permit fishing of yellowfin associated with dolphins in 1976, provided estimates made in the first quarter of the year indicated that the total 1976 dolphin kill would be less than 70% of the 1975 kill. The announcement raised a storm of protest from environmentalists, who went to court to try to force sterner measures on the industry. On May 11, 1976, Judge Richey in the Federal court in Washington DC set the tuna industry back on its heels.

In an unprecedented decision which minced few words, Judge Richey ruled that the Commerce Department's proposal violated the MMPA. The act, he said, places the interests of marine mammals above the economic interests of the tuna industry, and he ruled that all killing of dolphins by US fishermen must cease by the end of the month. The prohibition, he said, should stay in effect until the Department of Commerce can determine "that such killing is not to the disadvantage" of dolphin stocks. The decision was upheld by the Court of Appeals, but a delay in implementing it was granted until January 1, 1977.

In the meantime, the Department of Commerce came up with some new regulations for 1976. It said that tuna fishermen could continue to fish for yellowfin associated with dolphins until the total dolphin kill for the year reached 78,000. That level was reached on October 19, and after the tuna industry had exhausted its legal appeals, all killing of dolphin by US fishermen supposedly stopped in mid-November.

Conference of experts

In July, the department called a conference of experts in marine resources to try to ascertain present population levels of various dolphin species. The panel was asked to determine whether any species is in danger of falling below the so-called optimum sustainable population (OSP) level, and to try to estimate the impact on each species of various levels of kill next year.



Tuna fishermen at work

The conferees reported that the spotted dolphin population—the species most often caught in tuna nets—has been reduced to about 64% of its original level since the introduction of the purse seine. The eastern spinner dolphin—one of a number of types of dolphin which twist in the air when they jump out of the water—was reckoned to be in the worst shape; it has declined to nearly 50% of its original population level. The conferees said that a 50% decline in the population of any species would put that species below its OSP level.

One of the conferees, Dr William Aron, Director of the Office of Ecology and Environmental Conservation in the National Oceanic And Atmospheric Administration, testified last month that "while I do not contend that any of the (dolphin) stocks under consideration are presently endangered or threatened with extinction, past history indicates that a cautious approach is warranted". Aron argued that the unhappy experience with whale populations "suggests that a reduction in cetacean populations to much under half their original stock size may present a significant threat to survival of those populations".

On the basis of the conference report, the Department of Commerce issued in October regulations for 1977 which are designed to ensure that the number of animals of each species of dolphin will increase or at least remain within their OSP levels, an objective consistent with Judge Ritchie's ruling. The department essentially took the maximum kill levels which would still allow numbers of each species to increase, estimated the likely kill by non-US fleets, and set quotas for US tuna fishermen accordingly. The proposed regulations sent the tuna industry running for its lawyers and, if the industry's figures are accurate,

fighting for its survival. The industry is appealing the regulations in a lengthy public hearing.

The regulations would limit the total dolphin kill next year to some 61,400 animals, of which US fishermen would be able to kill some 29,900. In particular, the regulations would prohibit US tuna boats from setting their nets around dolphin schools containing any spinner dolphin.

Welcomes and worries

The proposed regulations have been welcomed by environmentalists. Christine Stevens, secretary of the Society for Animal Protective Legislation, calls the proposed quotas "a tremendous step in the right direction", though she points out that they are still some way from the MMPA's goal of insignificant mortality. And environmental groups, led by William Butler of the Environmental Defense Fund, have generally argued in favour of the proposals in the public hearings.

Nevertheless, the regulations will clearly have a severe economic impact on the industry. Even the Commerce Department's environmental impact statement on the proposals notes that the effect "may be severe". Unless the industry can reduce the number of dolphins killed per ton of tuna, the impact statement suggests that the US catch will drop by about one-third, which "could lead to the collapse of the US fleet if exvessel prices do not rise proportionately".

Industry spokesmen are now less restrained in their views. Frank Alverson, vice-president of Living Marine Resources, a research organisation supported by the tuna industry, claims that the total prohibition on netting spinner dolphin alone will "automatically deny the US fleet access to 68,000 tons of fish", valued at about \$45 million. That figure represents the

amount of yellowfin caught this year in association with spinner dolphin.

Environmentalists tend to take some of the industry's dire warnings with a pinch of salt, however. In particular, Butler has pointed out that tuna taken in association with dolphin actually account for a relatively small fraction of the total tuna catch. The Japanese tuna fleet, which is one of the largest producers, does not fish on dolphin at all. And a study done last year by Gordon Broadhead, president of Living Marine Resources, found that nearly 60% of the US tuna catch in 1975 consisted of skipjack or yellowfin caught when swimming in schools separate from dolphins. It is therefore argued that the US fleet will simply turn its attention to those other sources of tuna. Industry spokesmen counter, however, that such a switch would send the US fleet into areas already heavily fished by foreign vessels, and they argue that next year's skipjack catch is likely, on the basis of past trends, to be below average.

The industry therefore claims that the regulations will cause heavy losses among tuna boat operators, many of whom are already on the verge of bankruptcy. The industry is therefore fighting the regulations on several fronts. One line of attack is to challenge the estimated population levels, particularly that for the eastern spinner dolphin. Another is to argue that the regulations will penalise US tuna fishermen while allowing foreign fleets to fish at will.

Troublesome problem

The problem with non-US fleets could be troublesome. There is no mechanism available to reduce dolphin kills internationally, and thus, in meeting the

court requirement that there should be no detrimental impact on dolphin stocks, the Department of Commerce had to focus its attention entirely on the US fleet. The upshot is that if the regulations are put into effect, US fishermen will be denied access to tuna which could be available to other fleets and if the effect is to increase foreign fishing on dolphin, the net result could be disastrous.

The Department of Commerce estimates that the kill rate among foreign vessels fishing on dolphin is more than twice as high as the kill rate of US vessels. Thus, if foreign vessels move in on yellowfin schools denied to US vessels, the net effect could be to increase dolphin mortality.

There is, however, a way in which the United States government could exert leverage to prevent such a situation. The US is now the world's chief importer of tuna—domestic supply accounts of only about half US consumption. The MMPA gives the government power to insist that imported tuna is caught in accord with US regulations, and if such a requirement is enforced, a large switch in the operation of foreign fleets may be avoided.

The industry is also arguing that it has made considerable progress in reducing the mortality of dolphins per ton of tuna, and that if it is allowed to continue fishing, further progress can be anticipated. For years, tuna vessels have used a procedure known as backing down, which essentially causes part of the net to drop below the surface, enabling most of the dolphin to swim free. Another improvement, the so-called Medina panel, consists of a strip of fine mesh around the top of the net to reduce the chance that dolphins

will become entangled in the webbing, and other gear alterations are under test. According to Alverson, the tuna fleet has so far spent about \$1.7 million in gear modifications to reduce dolphin mortality.

Nevertheless, there is good reason to believe that the industry has not been as generous in funding research as it likes to suggest. It was not until December 1975, three years after Congress passed the MMPA, that the industry established a research body to study methods to reduce the dolphin kill. Known as the Porpoise Rescue Foundation, it is being funded only to the tune of \$250,000 a year. The federal government is funding at least three times that amount of research.

Though most of the studies are concentrating on gear modifications, two projects are focusing on different approaches. One, which is being tested on captive tuna in Hawaii, consists of trying to separate the tuna from dolphins by chemical attractants, and the other, which is much further from field testing, attempts to do the same thing by acoustical means.

Whatever the success of those methods in reducing dolphin mortality, it is clear that the industry will not be able to comply next year with the MMPA's goal of near zero levels. Consequently, the Department of Commerce will be hard put to justify relaxing its proposed quotas. The result, forecasts Lewis Regenstein of the Fund for Animals, is that the industry "will be breaking down the doors of Congress" to seek legislative relief. It will be a tough fight, but if the tuna fishermen get their way, environmentalists are already talking about the possibility of a boycott of tuna. □

White hope or white elephant?

What is happening at the European Molecular Biology Laboratory? **Walter Gratzer** looks at its first annual report

COMING at so bleak a juncture for European science, the 1975 annual report of the European Molecular Biology Laboratory (EMBL) strikes a brave and heartening note. To readers in Britain, who have seen the traditional patrons and defenders of molecular biology, the Research Councils, cringing under the lash first of Rothschild and now of the Treasury, a refreshing feature of the report is that it makes no effort to propitiate the avenging taxpayer: not once in its 50 pages is there even a token mention of heart disease, tooth decay or lower back pain.

The laboratory was conceived at the height of what Stent has called the Golden Age of molecular biology, when it was bliss to be alive, and its ten-year gestation period has seen many changes. A document published in 1966, when the project was still in the womb of time, asserted that for the undertaking to be worthwhile a multidisciplinary structure would be essential, and that all major areas of molecular biology would have to be represented; so grand a design, it suggested, would be outside the compass of any single European national institution. The laboratory would be a

major centre of scientific excellence and a nucleus for postdoctoral training, and it would nourish the university departments of Europe with a supply of the kind of young men then appearing in such profusion in America—minted at Harvard or MIT, and finished to a high radiance at NIH or Stanford. In this way Europe would regain the initiative that had by then passed to the Americans. The EMBL therefore must be large (150 scientists, 15–20 of them permanent). In contrast to CERN its object would not be to provide plant too expensive for the individual member countries to set up; in biology, the report affirmed, the plant consisted of interacting groups of scientists with outstanding and complementary talents.

Well, circumstances, as they say, alter cases and as Thurber observed, there is no safety in numbers or in anything else. The projected size and

scope appear both now to be considerably shrunk, and the report emphasises throughout the development of technical facilities sufficiently advanced to attract or generate interesting research projects. The laboratory is of course still in an early stage of growth, and is indeed a tenant in another institute in Heidelberg, pending the completion of its new building. Its Director is Sir John Kendrew, who has carried the entire project since its inception, and has recruited a predominantly young and active staff, but none of the ageing mandarins of the European molecular biology establishment.

Trendy blend

There is a trendy blend of research topics, with a strong cellular emphasis. The new investor, assembling his first portfolio, is of course best placed to take account of the steady rise in neurones and cell motility futures for example, without the pain of first getting out of slumping commodities, such as ribosomes or bacteriophage. The laboratory, then, has groups working on membranes in viruses and mitochondria, a division of biological structure (for the present confined to the development of methods in scanning electron microscopy), and a large division of cell biology, in which are subsumed four varied areas of research. There is a group working on chromatin and another on the control of chromosomal activity, using as the experimental material the giant chromosomes of insect salivary glands. There is a programme on control of morphogenesis, involving the isolation and study of peptide hormones that regulate differentiation in *Hydra*. An imaginative departure, not perhaps without the danger of creating an island within the laboratory, is the inclusion of a programme under Dr N. Strausfeld, concerned with the neuro-anatomy of the visual system of insects.

In addition to the activities in its Heidelberg laboratory, the EMBL has absorbed two major existing projects which in fact seem now to constitute its most substantial undertakings. These are facilities for X-ray diffraction with high-intensity radiation from a synchrotron and for neutron diffraction, and are based respectively at Hamburg and at Grenoble. These outstations are operated as a service to European workers, and are being furnished with supporting laboratories for preparative and related work. The neutron source has already been put to widespread use, and (at an estimated cost, as I am told, of \$1 per scattered neutron) provides an example of an installation that could nowadays scarcely be run on any but an international basis. The Grenoble outstation is directed by a member of the

laboratory staff, Dr A. Miller, and is evidently a thriving concern.

The synchrotron X-ray source in Hamburg was developed and delivered into the care of the EMBL by Professor Ken Holmes, and its potential is undeniably enormous. Some spectacular exercises have already been performed, and a number of diffraction photographs of truly remarkable quality from insect muscle have made the rounds of the conferences, and have apparently sent the adrenalin coursing through the veins of fibre crystallographers. By following the evolution of a single reflection on addition of ADP to a muscle fibre, it has even proved possible to obtain a titration curve, and from it a binding constant. By making use of the larger flux from a storage ring, also in Hamburg, further advances are anticipated.

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What questions will be answered with the aid of the new diffraction technology is not yet clear. However the report suggests that time-resolved diffraction in the millisecond range may soon become a reality, and with it structural stopped-flow experiments. The system most likely to yield to such an approach is obviously the cross-bridged cycle of muscle, but it is perhaps a valid article of faith that a technique so potent will foment other interesting applications.

Sober document

A few purple passages aside, the report is a sober document that offers few hostages to fortune; it is a pity though that it has come so late and gives no financial details. Many other interesting questions are left unanswered. The report lists a scientific staff of fourteen, a large proportion of them German, and some twenty visiting workers and research fellows. The final pattern of the laboratory has therefore yet to emerge. One wonders, however, where the young postdoctoral workers who are supposed to be the immediate beneficiaries of the institution are to come from. Not presumably from America, for then the EMBL will lay itself open to the charge, often levelled (however unfairly) at the MRC's Laboratory of Molecular Biology in Cambridge: that its unique intellectual

resources have been used to train American rather than indigenous workers.

And if it is to cater for Europeans, who will provide financial patronage? The number of fellowships available in Europe can be counted on rather few fingers, if one excludes EMBO. On the other hand the laboratory would be defeating the aims of its parent body if like a growing cuckoo in the EMBO nest it were to claim an increasing proportion of each year's fellowship allocation, at the expense of the very laboratories around Europe that the fellowship programme was designed to help.

What is reassuring at all events is that the report stands by the principle of the financial independence of EMBL and EMBO. There is therefore presumably no danger that, whatever economic hardships may lie ahead, the laboratory will ever be driven to continue its planned expansion at the expense of the fellowship programme. For nothing as felicitous as the EMBO fellowship scheme has emerged to invigorate European science in many a decade. It was conceived in the clear-sighted conviction that European biological science can become greater than the sum of its parts only through expansion of the scientific community, which in turn demands the kind of fluidity that has long been one of the bases of the American success. The impact of the EMBO fellowships has already been prodigious, and the Founding Fathers of the programme must be aware that it will be remembered in their favour on the Day of Judgement.

The first annual report of the EMBL suggests that as good a start has been made as could have been hoped. There seem at the moment no grounds for fears, occasionally articulated by the churlish in the early days of the project, that the laboratory would grow into a *monstre sacré*, which would consume too much of the limited pool of funds and talent available to the university departments. Indeed, in such a time of blight, it is opportunity rather than talent that is in short supply, and the additional jobs that the laboratory is to provide will be welcome.

As to whether the EMBL will be a success at the level at which it was conceived—as the Oxford historian is supposed to have said when questioned about the effects of the French Revolution, it is too early to tell. All biologists in Europe, and for that matter America, will wish to congratulate Sir John Kendrew on getting this courageous project launched. Many will see its progress as a barometer of the prevailing intellectual climate, and all, no doubt, will wish it well. □

IN BRIEF

Nuclear committee move

Democrats in the House of Representatives last week hammered a large nail into the coffin of the Joint Committee on Atomic Energy by voting to strip the committee of its legislative power. The move, if upheld in the full house when it reconvenes in January, would break the Joint Committee's thirty-year monopoly over nuclear legislation, and it would place nuclear matters in the hands of the committees which are likely to be more critical of the nuclear programme. The vote in the House Democratic Caucus, led by Representative Jonathan Bingham, would divide the committee's legislative authority among the Science and Technology Committee, the Commerce Committee and the Interior Committee, leaving the Joint Committee as a deliberate body with no power to handle

legislation. Similar moves will be discussed in the Senate next month as part of a complete overhaul of the Senate committee system.

Environment Ministers meet

As widely expected, the Council of Environment Ministers of the European Community, meeting in Brussels last week, could not agree on Commission proposals for dealing with the discharge into the aquatic environment of waste from titanium dioxide and paper pulp plants. The difficulty, as on similar issues before, concerned whether controls should apply to emissions directly or indirectly to the environment receiving them. The Council was able to agree to a new five-year (1977-81) environment programme focusing on air, water and noise.

UK-USSR agreement

Anglo-Soviet cooperation on environmental protection is to be extended. The third meeting of the joint UK-USSR Committee on Cooperation in the Field of Environmental Protection, held earlier this month in London, approved a plan for 1977 which includes the establishment of a working group on nature conservation, the setting up of a staged programme on urban transport and the rehabilitation and maintenance of residential areas, a work programme on land reclamation, and a study of possible cooperation regarding gas cleaning and dust elimination. The prevention and elimination of oil spills at sea is to be the subject of feasibility study. Topics are selected not on a basis of absolute priorities, but according to whether they produce a valuable sharing of information.

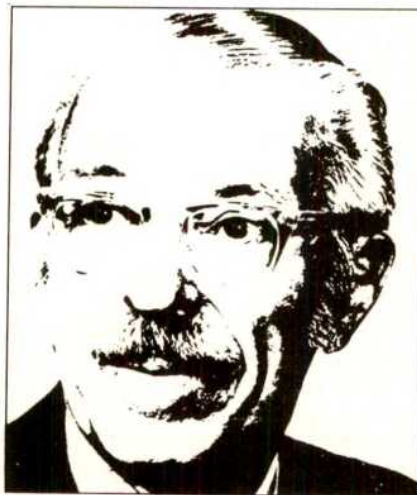
It is an old familiar story regarding industrial research that when sales decline, basic research is one of the first items to get the axe (rather than the President's plane). The justification is that accumulated knowledge is sufficient to keep things going for at least a while. In 1966, this philosophy became nationwide when President Lyndon Johnson was able to shift the federal support of science away from basic research and towards what is termed "mission-oriented research" or "research in the service of man". He said, for example, "We must make sure that no lifesaving discovery is locked up in the laboratory" (as if this were possible).

Professor Charles Tidball points out that the shift was made possible by anecdotal presentation of information to lawmakers. A factual examination of the background of "applied research" shows, however, that it is actually based largely on fundamental findings.

Decisions as to "what type" of science should be supported, or even permitted, depends increasingly on persuasive language, and today the language must be terse, so as to fit TV programmes. Slogans and phrases such as "environmental impact", "recombinant DNA", "carcinogenic chemicals", "endangered species", "noise pollution", "megavitamin therapy", and "world wildlife" are the current persuaders. They enable complicated matters to be put in neat little boxes that decision-makers can handle.

I was astonished to find recently

that one of the most popular persuaders of today, "food additives", is only eighteen years old. It did not become a category until the Food Additives Amendment of 1958. I regard the term, if you will excuse the cliché, as a pollution of the language, for most users of the phrase seem to think it means a class of substances. Actually

Language in action

THOMAS H. JUKES

it refers to a use to which diverse materials, many of them accepted foods, are put. There is a strong movement "against" food additives, and there is even an organisation "The Feingold Association", dedicated to their exorcism. The members are followers of an allergist, Dr Benjamin

Feingold, who has published a book (many MDs publish books instead of writing scientific articles) about hyperkinesis in children, and its relief, and that of their parents, by a diet containing no "food additives". I have not seen in any writings supportive of the "Feingold treatment" mention of the fact that numerous "natural ingredients" of food are more toxic than additives at levels of permitted usage.

It may be that Dr Feingold has found a way to rid harassed and inactive parents of that nuisance, the "hyperactive child", which, incidentally, is another slogan. But consider a partial list of what must be eliminated from intake or contact: apples, blackberries, cherries, peaches, cucumbers, pickles, tomatoes, ice cream, bakery goods (except "plain bread"), luncheon meats, jam or jelly, gin and all distilled drinks except vodka, tea, beer, wine vinegar, all soft drinks, aspirin, perfumes, toothpaste and toothpowder.

Is it possible that the goodies in this incredibly diverse list share any common biochemical or pharmacodynamic properties that make each and all of them inductive of "hyperactivity"? I find this postulation incredible, but millions of dollars will be spent to seek out the answer. I suggest that this expenditure is yet another example of "language in action".

Perhaps, however, sluggish adults should help themselves to tomatoes, ice cream, gin, toothpaste and perfume to induce hyperactivity.

news and views

Controversy over the extragalactic distance scale

from M. Rowan-Robinson

THE redshift-distance relation for galaxies discovered by Hubble and Lundmark in the 1920s is the most tangible evidence we have for an expanding universe. The constant of proportionality, the Hubble constant, can be interpreted as the expansion time-scale of the universe. Yet in spite of the consumption of many thousands of hours of telescope time, the extragalactic distance scale remains a subject of controversy, highlighted by a disappointingly inconclusive International Astronomical Union symposium at Paris in September.

At the symposium Tammann summarised the massive programme of galaxy distance measurements that he and Sandage have been pursuing, culminating in a value for the Hubble constant, H_0 , of around 50 km s^{-1} per Mpc (for a recent review see *Nature*, **262**, 97; 1976). Yet almost all other workers reporting at the symposium disagreed with the Sandage and Tammann distance scale, preferring a value for H_0 closer to 80. For example, van den Bergh and de Vaucouleurs reported significantly lower distances for members of the Local Group of galaxies, to which our Milky Way galaxy belongs. Hanes' work on globular clusters in galaxies in the Virgo cluster, the nearest of the great clusters of galaxies that determine the Hubble relation on the large scale, led to a distance estimate for Virgo of 12.5 Mpc (1 Mpc = 3.26 million light years) instead of the Sandage and Tammann value of 22. And Tully and Fisher used their 21-cm neutral hydrogen line method to obtain a value for H_0 of 75.

It is therefore fascinating to see in the latest *Astrophysical Journal* (**210**, 7; 1976) Sandage and Tammann's version of this last method. They take Tully and Fisher's data, reanalyse and recalibrate it, and emerge with a Hubble constant of 50.3 ± 4.2 . The 21-cm method depends on a correlation found by Tully and Fisher between the 21-cm hydrogen line

width and the optical luminosity of a spiral galaxy (one has to have some galaxies with distances known more directly to calibrate this relation). The apparent optical brightness of a galaxy then gives the distance through the inverse square law.

Sandage and Tammann use the method to obtain the distance of the Virgo cluster, and compare the result with six other methods (including, ironically, an earlier estimate by Hanes which agreed with theirs). The adopted Virgo distance, 22 Mpc, is then combined in three different ways with the mean velocity of the cluster

to yield $H_0 = 50.3 \pm 7.0$, 50.3 ± 4.2 , 50.3 ± 4.2 . The authors remark "That the values of H_0 from all three methods are the same is clearly fortuitous". If the agreement between these three means at so high a significance level is indeed simply good luck, then heaven protect these authors from bad luck.

Do these disagreements over H_0 , which appear even when the same data is used, matter? Well, first, the actual value of H_0 matters to cosmologists, because by comparing it with the age of our own and other galaxies they can deduce how long

Precise Lamb shift interval

from Peter Knight

FOR two years now, we have had two slightly different theoretical values for the hydrogenic $n=2$ Lamb shift interval. The more recent value, due to P. J. Mohr (*Phys. Rev. Lett.*, **34**, 1050; 1975) is $1057.864 \pm 0.014 \text{ MHz}$, and should be compared with the earlier value of $1057.912 \pm 0.011 \text{ MHz}$ due to G. W. Erickson (*Phys. Rev. Lett.*, **27**, 780, 1971). Published experimental values up to 1975 lacked the ultra-high resolution needed to support one value rather than the other. The 100 MHz natural linewidth of the Lamb transition imposes severe limitations on precision experiments, and great care is needed in the lineshape analysis.

At Harvard, S. R. Lundeen and F. M. Pipkin (*Phys. Rev. Lett.*, **34**, 1368; 1975) used a Ramsey-type separated-oscillatory-field technique combined with a fast atomic beam to achieve resonance widths of one-third the natural width, and an improvement of a factor of 3 in the precision of the experimental value for the $n=2$ hydrogenic Lamb shift, obtaining a value of $1057.893 \pm 0.020 \text{ MHz}$. Unfortunately this still overlaps with and is consistent with both theoretical values. This situation and future prospects were

recently reviewed by Pipkin (*Comments Atom. molec. Phys.*, **5**, 45; 1975).

More recently D. Andrews and G. Newton at the University of Sussex report the results of a new high-precision single-loop experiment, which whilst consistent with the Harvard experiment, manages to discriminate in favour of the new value of Mohr. The Sussex experimental value is 1057.862 ± 0.020 (*Phys. Rev. Lett.*, **37**, 1254; 1976).

It is unlikely that the precision of the Sussex technique can be improved significantly, since (as they say) they are at the limits of available microwave technology. Similarly the lineshape analysis of the separated-field approach is complex. One is, after all, trying to resolve a line centre to better than a few parts per million, when the fundamental natural width is as large as one-tenth of the resonance frequency. Opportunity for further progress seems to rest with the theoreticians. The source of the discrepancy between Mohr's and Erickson's theoretical values is unknown, but presumably is connected with the different ways of computing the self-energy contribution to the Lamb shift.

galaxies took to form. The big bang picture would be in trouble if the expansion time was less than the age of our Galaxy. Second, discrepancies which are such a large multiple of the quoted errors do not enhance the status of observational astronomy. At the moment the onus seems to be on the opponents of the Sandage and Tammann scale to assemble a similar body of observational data. Theoreticians will have to maintain the scepticism about H_0 that the wiser among them have always shown in the past. □

RNA and generation of positional information

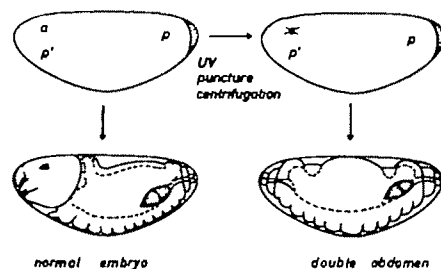
from Peter Lawrence

MOST biochemical investigation on developing systems is just conventional biochemistry on a system which happens to be developing, and is not relevant to the understanding of development in terms of the controlling molecules involved. (After all, even developing animals have to make proteins, send acids scooting round the Krebs cycle and so on). Conversely, specifically developmental phenomena, such as regulation are difficult to approach from a biochemical point of view. But several insects, including the tiny dipteran midge *Smittia*, undergo what Sander (*Wilhelm Roux Archiv.*, 167, 336; 1971) has called "negative regulation". An embryo regulates by reacting to experimental damage, such as removal of tissue, by respecification of cells so that normal development is restored. In negative regulation, however, respecification proceeds abnormally so that a monster develops. These striking changes in *Smittia* have provided investigators with a handle on the molecular mechanisms which may be involved in specifying that pattern.

Normally the insect consists of a number of segments arranged in order from head to tail; each segment is uniquely patterned. The segment order in most insect eggs becomes defined at blastoderm (when the embryo is just a hollow ball of cells) and depends only on the positions of the cells forming at the surface of the egg, not on the lineage of their nuclei. There is thus positional information in the egg. Experiments on other insects (see Sander, *Ciba Foundation Symp.*, 29, 241; 1975) suggest that the positional information is in the form of a gradient which is made by an interaction between two determinants, an anterior and a posterior one, at the poles of the egg. Kalthoff working in Sander's

laboratory (Kalthoff, *Roux. Arch.*, 168, 63; 1971) discovered that local ultraviolet irradiation of the anterior pole of cleaving *Smittia* eggs (the nuclei were untouched by irradiation) caused up to 100% of the embryos to change their segment pattern from 123456789 to 98766789 (Fig. 1). This aberrant pattern is called a 'double abdomen'. The targets to ultraviolet were localised in the egg cytoplasm and distributed unevenly in the anterior third of the egg, becoming most concentrated at the anterior tip. The damage done by ultraviolet was not violent: nuclei subsequently entered the irradiated region and developed normally into cells. Only the positional information, and hence the segment pattern, seemed to be altered. Kalthoff found the ultraviolet damage could be repaired by visible light so that normal embryonic development was restored; this suggested that nucleic acid might be the target. Action spectra (Kalthoff, *Photochem. Photobiol.*, 18, 355; 1973) showed peaks in both the protein and the nucleic acid regions.

This line has now been taken one step further by Kandler-Singer and Kalthoff (*Proc. natn. Acad. Sci.*, 73, 3739; 1976). They were able to produce double abdomens by gently puncturing the anterior pole of the egg in water containing RNase ($0.8 \mu\text{g ml}^{-1}$). Although about half the embryos failed to develop at all, 47% of those which did made double abdomens. In water only some 5% failed to develop and none grew up with double abdomens. With an elegant control they demonstrated that it was actually the RNase activity which was responsible for altering the segment pattern. They treated the eggs with two fragments of RNase each of which is inactive separately (RNase S (active) is split by TCA fractionation into S-peptide (inactive) and S-protein (hardly active)). When solutions of the two fragments are mixed RNase activity returns. The percentage of double abdomens was nil with S-peptide, 1 with the S protein



Diagrammatic representation of the induction of a double abdomen in *Smittia* eggs by ultraviolet irradiation of the anterior pole region, puncture at the anterior pole or centrifugation with the long egg axis parallel to the centrifugal force. (From *Insect Development*, edited by Lawrence, P., Blackwells Scientific, Oxford and London, 1976.)

and about 40 both with complete RNase S and with the two fragments dissolved together. Puncturing of the eggs elsewhere than at the anterior pole produced no double abdomens. Double abdomens were formed only when the experiment was performed up to the blastoderm stage of embryogenesis. These results together with those mentioned earlier, strongly suggest that an RNA moiety is an essential component in the generation of positional information in the egg. Kandler-Singer and Kalthoff suggest it might be mRNA that is translated during cleavage.

The next stage in this investigation will ideally be the development of a positive assay in which the damage done by ultraviolet will be repaired by the injection of extracts into the anterior pole of the egg. This could lead to the first isolation of a molecule specifically involved in the generation of positional information and perhaps to some inkling as to what gradients are in molecular terms. □

Structural gene for sex pilin

from J. P. Beard

A RECENT paper from Charles Brinton's laboratory in Pittsburgh reports on an important aspect of the mechanism whereby 'male' Gram-negative bacteria are able to transfer genetic material to 'female' bacteria by conjugation. This subject has been of great interest to microbiologists for many years, since not only does the conjugation process provide a useful research tool for probing the genetic composition of *Escherichia coli* and related species, but it is also usually mediated by genes carried on plasmids in the male or donor cell. Plasmids are small extrachromosomal pieces of DNA, carried by a wide variety of bacterial genera, which are capable of autonomous replication in the host cell, and which can often transfer both themselves and chromosomal DNA in the conjugation process. Plasmids are also important as vectors of multiple antibiotic resistance genes, or of genes which confer other important properties on the host such as colicinogeny or enterotoxin production (see, for example, the *Ciba Foundation Symposium on Bacterial Episomes and Plasmids*, J. and A. Churchill, 1969), and have recently taken on a new lease of life as vectors for "genetic engineering", currently the subject of intense public discussion.

Studies of one such plasmid, the sex factor F (Hayes, *The Genetics of Bacteria and their Viruses*, Blackwell,

1968), have shown that male, or F^+ , cells produce filamentous appendages on their surfaces—sex pili, which are required for both conjugation and sensitivity to male-specific bacteriophages. In addition, it has been demonstrated that sex pili are composed of a single protein, sex pilin, of molecular weight 11,400, which contains one mol of glucose and two mol of phosphate per mol of pilin, while genetic studies have shown that the transfer process is controlled by at least twelve genes on the plasmid whose order has been established by deletion mapping to be

traJ traA traL traE traK traB
traC traF traH traG traD traI

It has been shown that the product of the first of these genes, *traJ*, is required for the expression of the other genes, *traA* to *traI*, which constitute a single operon (Willets, In *Microbial Drug Resistance*, edit. by Mitsuhashi and Hashimoto, University Park Press, Baltimore, 1975; and Helmuth and Achtman, *Nature*, **257**, 652; 1975). Mutants in any of the first nine genes (*J. A. L. E. K. B. C. F* and *H*), and some in the tenth (*traG*), lack sex pili, and complementation analysis of a range of the mutants in the presence of another F -like plasmid (R100-1), which specifies slightly different sex pili, suggested that *traA* was a likely candidate for the F pilin structural gene (Willets, *Nature new Biol.*, **230**, 183; 1971). This has now been confirmed in a series of elegant experiments by Brinton's group (Minkley, Polen, Brinton and Ippen-Ihler, *J. molec. Biol.*, **108**, 111; 1976) in which they took advantage of the fact that sex pilin contains only two tyrosine residues per molecule. By using amber (nonsense) mutants in the *traA* gene which were originally isolated by Achtman *et al.* (*J. Bact.*, **106**, 529; 1971) and a *supF* host strain which suppresses amber mutations by inserting a tyrosine residue at the amber site during protein synthesis, Minkley *et al.* have been able to isolate the sex pili specified by the mutant plasmid in the suppressing host. Subsequent iodination of the pilin protein derived from these sex pili, using radioactive iodine-125, and preparation of peptide maps (2-dimensional separation on thin layers of cellulose) after tryptic digestion, has revealed three radioactive spots in the case of tyrosine-suppressed *traA* sex pilin compared with two radioactive spots from pilin obtained from serine-suppressed *traA* sex pili (produced by a *supD* host carrying the *traA* mutant plasmid).

This neat identification of *traA* as the structural gene for sex pilin is a first step towards a detailed biochemical analysis of the biosynthesis of sex pili, and it should form the basis of a clearer understanding of the roles of the re-

maining *tra* genes in the processes of conjugation and male-specific phage infection. In the long term, this work could provide molecular biologists with a unique model for study of a complex surface polymer, specified by an easily-manipulated plasmid genome, whose biosynthesis and assembly is likely to involve important interactions with other, host-specified surface components of the bacterial cell. □

Watchers of the skies

from David W. Hughes

NETWORKS of all-sky cameras are coming and going in modern astronomical research. Coming because the Russians are just installing an enormous new system in the south of the USSR. Going because, almost simultaneously, the American Smithsonian Institution is abandoning and dismantling its Prairie network.

Zotkin, Simonenko, Fedynsky and Khotinok (Meteorite Committee of the USSR Academy of Science) and Kramer (Astronomical Observatory of the Odessa University) discuss the Russian Network in a recent paper in *Meteoritika* (USSR Acad. Press, **35**, 3; 1976). This will have 39 camera stations separated by 180 to 240 km spaced throughout 900,000 square km of the River Don basin, the Ukrainian and Moldavian Republics and the North Caucasus. This is a relatively flat, densely populated part of the country with well-developed communication links. It is hoped that the network will provide about 100 photographs of fireballs brighter than -9.0 visual magnitude over a 5-year period. The velocity, time of appearance, atmospheric trajectory and previous orbit of each of the fireball-producing meteoroids can be obtained by careful analysis of the photographic plates. It is estimated that between 1 and 3 of the fireballs photographed will also be so bright that the causative interplanetary body will not completely ablate in the atmosphere and a part of it will fall to ground as a meteorite. The fireball's atmospheric trajectory enables the area of fall to be calculated and it is hoped that a detailed search of this region will lead to the recovery of the meteorite. This will not only increase the number of meteorites available for analysis but much more importantly will provide a special class of meteorites which have well-known orbits. Only three meteorites, Příbram, Lost City and Farmington are in this special category so far.

The Russian system will be the fourth in Europe. The first one to be set up was over the whole of Czechoslovakia (*Bull Astr. Inst. Czech.*, **16**, 15; 1965)

which has lately been joined by a similar network covering the south of the Federal Republic of Germany. There is also a large network in the British Isles run by the Meteor Section of the British Astronomical Association (*J. Br. Astr. Assoc.*, **85**, 150; 1975). In North America a Canadian system watches over southern Alberta, Saskatchewan and Manitoba. The old Prairie network (*SAO Special Report* No. 193, 1965) used to cover all of South Dakota, Nebraska, Kansas, Oklahoma, Mississippi, Iowa and Illinois. It was hoped that this Prairie network would lead to the recovery of at least one meteorite per year but unfortunately this was not to be. Many fireballs were photographed and our knowledge of these awe-inspiring objects increased enormously as a result of the data collected but paradoxically it was found that few brilliant fireballs actually lead to meteorite falls. The Lost City meteorite was the only one found during all the years of operation.

A second use of these networks has recently come to light. At the Ondřejov Observatory near Prague, Czechoslovakia, an Opton-Distagon fish eye objective on a large (9×12 cm) plate camera is used to photograph systematically the entire celestial hemisphere each moonless clear night. This has been done since March 1975 when a programme was started to replace the old 36 cm convex mirror-type all sky cameras previously used throughout the Czechoslovak network by these newer cameras. The image quality produced by this fish-eye lens is excellent. The camera is driven and with the usual 4 h exposure, reaches a magnitude limit of +11 and has a resolution of 1 min arc. The changes in image quality as far as 70° from the centre of the field of view is still small enough to be removable by small correction terms. Boček, Cepelch, Ježková and Novák used the camera to take a set of 13 photographs of Nova Cygni 1975. The image definition was so good that there was an almost linear dependence of star image diameter on stellar magnitude. Over 20 comparison stars could be used on each plate, all at a zenith distance of less than 40° and with comparative ease the visual magnitude (*V*) of Nova Cygni could be obtained to a precision of ± 0.10 magnitudes. These observations, made between August 31.8 and September 2.9, are reported in the latest edition of the *Bulletin of the Astronomical Institutes of Czechoslovakia* (**27**, 190; 1976).

With the ever increasing use of these sophisticated cameras throughout Czechoslovakia it is hoped that, as well as leading to meteorite finds, the

rise in brightness of new novae can be accurately plotted as well as the decay. With a limiting magnitude of +11 it is hoped that many more than the 2.2 novae normally discovered each year will be found. □

Probing specific translational controls

from Martin Blundell

In principle, the expression of genetic information may be regulated either at transcription or at translation. Control of transcription eliminates the synthesis of unnecessary messenger RNA but control of translation could act more quickly, particularly in eukaryotic cells, where messenger half-lives are measured in hours.

Most control seems to be exerted at transcription or possibly (in eukaryotes) during the processing and transport of mRNA from the nucleus. Nevertheless, translational control is clearly established in eukaryotic cells. In reticulocytes, for example, the translation of globin and other mRNAs is controlled by diverse agents such as hemin and double-stranded RNA. Other cells respond in the same way to virus infection and alteration of their growth medium. In general, this sort of translational control appears rather unspecific. Thus, haem deficiency shuts off all reticulocyte protein synthesis, not just the synthesis of globins.

Translational controls seem more difficult to detect in *E. coli*, where message half-lives are much shorter (even when expressed as a fraction of the doubling time of the cell). Translational controls do exist, however, and they may be more specific than those in eukaryotes.

When *E. coli* is infected by phage T7, a T7 gene makes a translational repressor which switches off host protein synthesis (Herrlich *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1088; 1974) presumably to maximise production of T7 proteins. Another kind of translational control is seen during infection by phage λ (Ray and Pearson, *J. molec. Biol.*, **85**, 163; 1975; *Nature*, **263**, 647; 1976). The late genes of λ are all transcribed at about the same rate, but the number of protein molecules made from different genes varies nearly a thousand-fold. Since the functional lifetimes of the various messages are all about 2½ min, the differences in quantity of protein observed can only be explained by varied efficiencies in translation or possibly by differences in the stability of the proteins themselves.

In uninfected *E. coli* there seems to be a control of translation of some inducible messages. In three rather different cases, the translation of β -galactosidase message is impaired when general protein synthesis appears normal. Ennis and Kievett (*J. biol. Chem.*, **251**, 2854; 1976) have studied cells recovering from inhibition of protein synthesis by either chloramphenicol or deficiency of potassium ions (the latter avoids difficulties which might arise from residual antibiotic). In these cells, *lac* mRNA is made but is only poorly translated. Other proteins as large as β -galactosidase and larger are being made, so the effect is not just a lesion in protein synthesis. The message can function *in vitro* to make the amino-terminal part of β -galactosidase, but not even this part is made *in vivo*. Although more complex explanations remain possible, the simplest is that some control on translation is operating in the recovering cells. The same authors also report under-translation of *lac* mRNA in *lac* O^c mutants and *trp-lac* fusion strains (*J. Bact.*, **125**, 1220; 1976).

The most recent work of Herrlich and Schweiger (*Proc. natn. Acad. Sci. U.S.A.*, **73**, 3386; 1976) suggests translational control of a number of inducible genes. The main thrust of their paper is to determine the mode of action of nitrofurans, a class of synthetic antibiotics in widespread use as food additives and in medicine. When low concentrations of nitrofurans are added to cultures, the synthesis of inducible proteins (β -galactosidase, tryptophanase, galactokinase and others) is inhibited while other proteins are unaffected. The possibility that this effect is mediated by cyclic AMP and catabolite activator protein is eliminated.

Three lines of evidence indicate that the drug acts on translation. First, in EDTA-permeabilised cells, nitrofurans act on β -galactosidase synthesis more rapidly than do rifampicin or actinomycin. Second, in strains where *lac* transcription is fused to nitrofuran-resistant genes (either the *trp* operon or *lac i^a*) β -galactosidase synthesis remains nitrofuran sensitive. However, when a deletion fuses the peptides made by the *lac i* and *lac z* (β -galactosidase) genes, the production of the fusion peptide is nitrofuran resistant. Third, *in vitro* synthesis of galactokinase is equally sensitive to nitrofuran whether directed by DNA or RNA, whereas *gal* transcription is unaffected.

If nitrofurans are to act specifically on the translation of certain proteins, there must be some recognition signal. The only stage at which recognition could easily occur is at the initiation of translation. This conclusion is supported by the nitrofuran-resistant

synthesis of the *lac* repressor: β -galactosidase fusion peptide and by a further experiment on galactokinase synthesis *in vitro*. When preformed polysomes are used for galactokinase synthesis, so that ribosomes have already initiated, nitrofuran no longer inhibits. Apparently a group of messengers carries a signal different from other messengers, and the translational machinery is able to recognise this signal. Such signals could presumably have evolved to provide specificity in a translational control system.

How much use does *E. coli* make of translational control? Unless a message is completely inactivated, translational control would generate polysomes with fewer ribosomes than usual, but electron micrographs (for example, *Cold Spring Harbor Symp. quant. Biol.*, Miller, **35**, 505; 1970) show that the density of ribosomes on mRNA is remarkably uniform. □

Plumes in an expanding Earth

from Peter J. Smith

THE expanding Earth hypothesis has been part of the geophysical backdrop for at least 20 years. Indeed, its origin in physics may be traced back even further, to Dirac (*Nature*, **139**, 323; 1937) who first proposed that the gravitational constant may not be a true constant but may be slowly decreasing with time. More recently, Steiner (*J. Geol. Soc. Aust.*, **14**, 99; 1967) has suggested that the gravitational constant may be varying cyclically rather than decreasing monotonically, but neither this idea nor Dirac's has received convincing proof. The point is, however, that during a period in which the gravitational constant is decreasing, the forces between particles would also be decreasing, and the volume of the Earth would therefore be increasing.

On the whole, Earth expansion has found little favour among geologists and geophysicists. It has a few very strong supporters, notably Carey (*Earth Sci. Rev.*, **11**, 105; 1975); and various workers have from time to time tried to test the hypothesis by, for example, palaeomagnetism (which can be used in theory to detect changes in the Earth's radius) and the fitting of continents onto globes with reduced radii. But such attempts have never been entirely successful, often because the data available were insufficiently accurate to resolve expansion of the Earth at low rates. The real reason for the expansion hypothesis's lack of success, however, is less the evidence against it than the

fact that it seems to be unnecessary. In other words, few are convinced that there are any major phenomena that cannot be explained by plate tectonics.

But this is far from saying that the expansion has not occurred. The question remains unresolved; and it is still open to anyone to test the hypothesis against any appropriate data. This is precisely what Stewart (*Geophys. J.*, **46**, 505; 1976) has now done using hot spots and mantle plumes. Of course, mantle plumes are as controversial as the expanding Earth. There are certainly hot spots at the Earth's surface, but whether they reflect underlying crustal-upper mantle phenomena or whether they are due to thin columns rising from the core-mantle interface is still a matter of debate.

One of the main attractions of mantle plumes, however, is the possibility that they might be laterally stable and thus provide a frame of reference fixed to the mantle. Evidence on this point is mixed, with some workers claiming to have shown that plumes remain fixed with respect to each other and others claiming to have detected relative motion. But as Stewart points out, all such exercises have been carried out assuming the Earth to have maintained a constant radius. So how do the data appear against the background of an expanding Earth?

To find out, Stewart has determined the great circle angular distances between the members of pairs of hot spots for three times—the present, 50 Myr ago and 120 Myr ago—assuming a constant terrestrial radius. For points within the same plate, the average ratio of the 50 Myr angles to the present angles is 0.98 ± 0.04 which is not significantly different from unity. The average ratio of the 120 Myr angles to the present angles, however, is 0.88 ± 0.03 . The errors on these figures are estimated to be up to 5%, largely because of the difficulty of locating accurately the centres of hot spots which may be 150 km or more across. But taken at face value the older figure suggests that the average great circle separation between members of hot spot pairs could have increased by 11–17% over the past 120 Myr and even up to 6% over the past 50 Myr.

One explanation of these remarkable results is that the hot spots are moving with respect to each other at relative speeds of $0.5\text{--}2.0\text{ cm y}^{-1}$ across the surface of an Earth with constant radius. But in that case it would be strange that the members of most pairs are moving away from each other, for in a random system one would perhaps expect as many hot spots to be converging as diverging. On the basis of Stewart's evidence, therefore, it seems much more likely that the geocentric angles between the supposed mantle

plumes remain roughly constant whilst the Earth expands to produce divergence of the hot spot locations at the surface.

As Stewart admits, his results can hardly be said to prove that the Earth has expanded. But they are a small step in that direction. □

Balancing the nutrient budget

from Peter D. Moore

THERE have been many commendable attempts to reduce to quantitative terms the influence of catastrophes on the flow of energy through and the cycle of nutrients within ecosystems. Some of the most carefully monitored experiments have come from the Hubbard Brook ecosystem studies in New Hampshire (for example, Likens *et al.*, *Ecol. Monogr.*, **40**, 23; 1970) and the Coweeta catchment area studies in North Carolina (for example, Swank and Douglas, *Science*, **185**, 857; 1974). In both these experimental areas, deciduous forest cover has been destroyed and the resultant changes in runoff water and nutrient losses have been observed. At neither of these sites, however, have the consequences of these hydrological and chemical changes been considered from the point of view of those ecosystems which receive the effluent generated by the catastrophe.

A study has recently been undertaken in Minnesota by Wright (*Ecology*, **57**, 649; 1976) in which the chemical impact of forest fire upon the nutrient budget of receptor lakes has been determined. The forested catchments of Meander Lake and Lamb Lake in the north-east of Minnesota were extensively burned in a serious fire in 1971. Changes in the nutrient budget and hydrology of these lakes were compared with those of Dogfish Lake, a similar site whose catchment lay outside the burned area.

The percentage increase in runoff attributable to the fire was 30% at Lamb Lake and 60% at Meander Lake. The latter value is considerably larger than values obtained after clear felling catchment areas at Coweeta and Hubbard Brook (15% and 40% increases respectively) and the reason for this may be the loss of the absorptive moss and humus layers after the fire in Minnesota. As it moves through the system, water may receive and may also lose various ions. On arrival at the ground, precipitation may move for a short distance over the surface, particularly if the soil is frozen. In burnt areas this water becomes enriched with phosphates. On percolating into the soil

The ecology of dragons: a reply

I AM glad that Robert May thought my article (Hogarth, *Bull. Brit. ecol. Soc.*, **7**, 2; 1976; May, *Nature*, **264**, 16; 1976) seminal; I must, nevertheless, disagree with him on one of his criticisms. In classifying the wyvern and cockatrice separately from the dragon, he attaches more significance to limb number than to other characteristics which these three types held in common. In particular, I refer to their supernatural qualities which seem to me to be of sufficient importance to justify setting them apart in a taxonomic category separate from that of the vertebrates. Four legs do not make a tetrapod; it is no more logical to include wyverns and cockatrices with conventional vertebrates than to classify a 12-legged dragon as a myriapod. Convergent evolution has indeed occurred to a remarkable extent, but between dragons and their allies on the one hand and vertebrates on the other. Not, as May suggests, between vertebrates (including the wyvern and cockatrice) and dragons.

My estimate of 5,000 years BP for the emergence of dragons is therefore feasible. The total dependence on human imagination for their existence (a unique ecological relationship) precludes an origin earlier than, say, the late Pleistocene. The absence of convincing representations of dragons in upper Palaeolithic art, and their frequent occurrence in literature and art from the time of Babylon onwards, indicates the chronological limits on the possible time of emergence. Between these limits, 5,000 years ago seems a reasonable, albeit imprecise, estimate.

P. J. HOGARTH

these phosphates are adsorbed onto soil particles, but the general cationic load of the water is increased. Stream water is considered to be equivalent chemically to this subsurface flow of water in the soil.

Wright determined nutrient budgets of phosphorus and various cations by monitoring input from precipitation and output in streamflow. The difference between these two rates is equivalent to the weathering input less any change in storage capacity in the system (including biomass growth). It is regrettable, though understandable, that these two components in the budget cannot be separated, but considerable additional measurements

would be necessary in order to achieve this. In the absence of such information, however, conclusions should be tempered with caution. For example, Wright states that nutrient cycling in the undisturbed forest is efficient, with only minor amounts leaking from the system. At the control site, Dogfish Lake, $13.7 \text{ mg m}^{-2} \text{ yr}^{-1}$ P arrives in precipitation and only 1.5 leaves in runoff. Rather than invoke the terms 'efficiency' or 'conservation' in such a situation, one should suspect an increasing storage capacity, probably due to biomass growth. If there were no storage change then inputs should be equalled by outputs, so the terms 'efficiency' and 'conservation' are not particularly informative in nutrient budget studies.

It is evident from Wright's data, however, that the output of nutrients at the burned sites exceeded expected values based on extrapolation from the control data. Such figures for increased discharge as 26% for Ca, 29% for Mg, 265% for K, 65% for Na and 93% for P were obtained from the Meander Lake catchment. Thus water entering the lakes had been substantially enriched in both phosphorus and cations.

Input of nutrients to the lakes is derived from runoff water and also directly from precipitation. At his control site Wright found that runoff was the major source of cations, but that precipitation accounted for over 80%

of the phosphorus input. This is presumably due to the adsorptive properties of the soils for phosphorus. At Meander Lake, after catchment burning, runoff inputs were increased yet further for the cations, and for phosphorus were brought up to the same level as that derived from precipitation. The total increase in phosphorus load at Meander Lake as a result of this change was 38%. In Wright's opinion this lies within the kind of year to year variation expected in phosphorus supply and does not represent a major eutrophication episode. If this is so, then the adsorption of phosphorus in the soil of the catchment is critical for the maintenance of stability of lakes in periods of catchment disturbance. □

Man's influence not yet felt by climate

from John Gribbin

THE message conveyed by Professor B. J. Mason, Director-General of the UK Meteorological Office, in a recent lecture was—don't panic. The theme of Mason's lecture (given to the Royal Society of Arts on December 1) was "Man's Influence on Weather and Climate", and his conclusion was that the climatic system is so robust, and contains so much inherent stability through the presence of negative feedback mechanisms, that man has still a long way to go before his influence becomes great enough to cause serious disruption to the natural climatic system.

Mason began his discussion by setting the influence of man in the context of the natural climatic changes that occur continually, pointing in particular to fluctuations of temperature in the United Kingdom during the three centuries for which instrumental records are available. In the past, fluctuations on the scale of 1°C variation in the annual mean temperature have had less impact on society than similar changes may have in the future; as Mason stressed, the present large world population and inadequate food reserves now put the agricultural system under unprecedented strain. "There is no question that climate is variable and that variations have a greater social and economic impact than ever before", so that changes of 1°C in the long term mean cannot be ignored—such a variation in the United Kingdom could, for example, change the length of the growing season by three weeks. But is man's influence yet approaching even this level?

Three anthropogenic influences have been widely cited as potential causes of climatic disaster: the "greenhouse

effect" caused by CO_2 from burning fossil fuels; cooling produced by dust in the atmosphere; and the effects of chlorofluorocarbons and supersonic aircraft on the stratospheric ozone layer. Mason's analysis of the problems hinged upon the computer modelling of the atmosphere which is the forte of the Met Office, and the numbers he produced were surprising, if reassuring, to at least some members of his audience. A dust layer in the stratosphere thick enough to cut off 4% of incident solar radiation, for example, warms the upper atmosphere by as much as $10\text{--}20^\circ\text{C}$, but produces no detectable effect on the troposphere in the models. And this conclusion may be borne out by events in the real atmosphere after the volcanic eruption in Bali in 1962, when the temperature of the stratosphere increased by some 6°C without any observable effects, according to Mason, on the lower atmosphere.

The ozone problem has been highlighted by controversy surrounding Concorde and spray cans, widely reported in *Nature*. Both the Met Office and American models have now shown that even a fleet of 500 SSTs flying for 5 hours per day each would not reduce the ozone content of the stratosphere by more than 1%, and although the hazard from spray can propellants seems greater, even there release of "freons" could continue at the present 700 kt yr^{-1} until 2100 AD before ozone would be reduced by 8%. Mason feels that the decision in the US to ban such propellants is over hasty, not least because if release at the present rate continued for another 5 years the climate modellers would have enough hard figures to produce better models of what is going on.

But the can is now being banned, at least in the US, and we are left with the release of CO_2 as man's only real, quantifiable contribution to climatic change yet or in the near future. Having cautioned that if the effect is real, something must have offset it in recent decades, since the Earth has actually been cooling down, not warming up, Mason suggested that within 50–100 years the effects of CO_2 release into the atmosphere will produce a warming of $1\text{--}2^\circ\text{C}$, well into the range significant for our present global society. Computer modelling indicates that rainfall patterns would be shifted by 5–10%, but the models are not yet sophisticated enough to detail the effects on specific regions of the globe. There is, says Mason, a real and significant effect here which must be investigated further with bigger and better models—but there is time to develop the necessary models and no need for panic induced by the prophets of doom. □



A hundred years ago

A NEW STAR IN CYGNUS. — On November 24, at 5h 41m P.M., the director of the Observatory at Athens, Prof. Schmidt, remarked a star of the third magnitude not far from ρ Cygni, which was not visible on November 20, the last clear evening previous. Its position from observations with the refractor was found to be in R.A. 21h. 36m 50.5s., N.P.D. $47^\circ 40' 34''$ for the beginning of the present year. At midnight its light was more intense than that of η Pegasi, which is rated a third magnitude by Argelander, and very yellow.

Direct intimation of this discovery was given by Prof. Schmidt to M. Leverrier, and the Paris *Bulletin International* of December 6 contains the few particulars concerning this star which the generally unfavourable weather up to that date had permitted to be put upon record. M. Paul Henry estimated it of the fifth magnitude, so that as in the cases of the similar suddenly-visible stars of 1848 and 1866, it would appear to have remained but a very short time at a maximum. He considered the colour "greenish, almost blue" by comparison with Lalande 42,304, not far distant.

From *Nature*, 15, December 14, 146; 1876

review article

Significant journals of science

Eugene Garfield*

In 1974 the Science Citation Index (SCI) covered about 401,000 articles and communications in 2,443 scientific and technical journals. They cited about 3.2 million different publications an average of 1.8 times each. In this article some results of an analysis of more than 5 million citations in the references of journal articles indexed for the SCI in 1974 are presented and an attempt is made to interpret of those results in the light of an earlier study of 1969 citations.

THE basic information recorded in the *SCI* for citing and cited papers is a "condensed citation." It gives first author, year, journal, volume, and page. The citing-cited pairs can be sorted and subsorted in various ways, as one's interests dictate. Sorting by cited author produces the *Citation Index* section of the *SCI*. Sorting by citing and cited journals produces the two major sections of the *Journal Citation Reports (JCR)*.

ISI's *Journal Citation Reports* is an index of journal-journal links based on a grouping and summation of condensed citations using journal rather than author as the primary sorting key. A preliminary *JCR*, based on an analysis of 1969 references¹, appeared in 1972². This year the *JCR* became a regular section and volume of the *SCI*³. It is the source of the 1974 citation data discussed here.

In this report I have used two indicators of journal significance: total citations and impact. The first is simply the number of times a journal was cited in 1974. Impact, on the other hand, is a measure of the relationship between citations and articles published. For this report, impact was calculated by dividing the number of 1974 citations of 1972 and 1973 articles by the number of articles published in 1972 and 1973. For example, the 817 articles published in 1972 and 1973 in the *Journal of Molecular Biology* were cited 6,129 times in 1974. The impact of the journal is therefore 7.502.

Fig. 1(a) lists the 206 journals most cited in 1974. Fig. 1(b) lists an additional 78 journals whose 1972 and 1973 articles only—rather than articles of any and all years, as in Fig. 1(a)—were highly cited in 1974. (The total of 284 journals in Fig. 1(a) and (b) corresponds to the number of journals listed in Fig. 2(a) and (b), which have impacts greater than 2.) In most cases (63%) these journals began publication in the 1960s and 70s. Older journals like the *Comptes Rendus* rank well in Fig. 1(a), mainly because there is so much that can be cited. Fig. 1(b) is a needed supplement to the list in Fig. 1(a), since the journals have high current citation but lack historical mass to push them up into the top of a list ranked by total citations.

Figures 2(a) and (b) show the 284 journals with impacts greater than 2. Fig. 2(a) lists 206 primary journals. Fig. 2(b) lists 78 review journals; the impact of review journals is generally higher than that of primary journals.

Figure 3 lists journals that rank highest in citation and

impact for three specialties: mathematics, botany, and astronomy/astrophysics. The differences in average impact and citation between the three illustrative categories indicate why comparisons between journals in different specialties may be invidious. For example, it would be foolish to conclude merely on the basis of citation counts that *Astrophysical Journal* is a "better" journal than *Annals of Mathematics*, or to hypothesize without a great deal of study which serves its own field "better."

Variation from field to field is determined by the interplay of several factors. Perhaps the most important is the average number of references per paper in the field⁴. In general, mathematicians cite less than half as many papers as do biochemists. Engineers on the other hand cite books as heavily as journals, as do social scientists. Furthermore, calculation of impact based on 1972 and 1973 publications is bound to affect the impact of journals in a field like mathematics, where citation of older literature is far more common than in others. Thus, the impact of mathematics journals would be higher if calculated on the basis of 1970 and 1971 publications.

It seems necessary to point out the obvious, as I have done in preparing Fig. 3, because one short-sighted criticism of the *JCR* has been that its listings and rankings are indiscriminating. One can get from the *JCR* information on journals within disciplines for intradisciplinary comparison. None of the mathematics journals listed in Fig. 3 was cited enough to appear in Fig. 1(a), but the citation counts and impact factors show plainly that the two leading mathematics journals are *Transactions of the American Mathematical Society* (on the basis of total citations) and *Acta Mathematica* (on the basis of impact). In both citation and impact the average mathematics journal ranks lower than the average astronomy or botany journal.

If one wishes to add to a general-science collection the two or three leading journals of mathematics, botany, or astronomy/astrophysics, one must examine longer lists and select from them the top journals in each specialty, as I have done in preparing Fig. 3.

The remarkable stability of the significant journals of science is attested by their continued high citation and impact. Of the 206 journals most cited in 1969, 169 remain among the top 206 in 1974. One may regard the changes as the result of healthy competition. The 37 journals that dropped from the 206 most cited between 1969 and 1974 rank between 224 and 426 in the complete listing that appears in the *JCR*⁵.

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Fig. 1a. Journals most highly cited in 1974. Journals are listed in descending numerical order of total citations in the references of 1974 issues of journals processed for the *Science Citation Index*. A: rank in terms of total 1974 citations. B: rank in terms of total 1969 citations. C: total 1974 citations. D: 1974 impact. E: total 1974 citations of 1972 and 1973 articles. F: rank in terms of 1974 citations of 1972 and 1973 articles. An asterisk before a journal title indicates that counts for sections, retitled continuations, translated versions, and so on, have been combined with those for the original; the number in parentheses after the journal title indicates the number of such sections, and so on, that went into the combination, including the original. b. Journals whose 1972 and 1973 articles were highly cited in 1974. Journals are listed in descending numerical order of total 1974 citations of their 1972 and 1973 articles. Journals ranking higher in this respect will be found among the journals listed in Fig. 1a. See the legend of Fig. 1a for significance of the column markers. An asterisk before a journal title indicates that counts for sections, retitled continuations, translated versions, etc., have been combined with those for the original; the number in parentheses after the journal title indicates the number of such sections, etc., that went into the combination, including the original. The date of a journal's inauguration follows its title.

a

A	B	C	D	E	G	H
1	1	98995	J. Am chem Soc	4 383	17088	3
2	2	91645	*Physical Rev (5)	2 670	19174	1
3	3	81353	J biol Chem	5 843	13685	6
4	5	75206	*Nature (3)	4 006	18924	2
5	4	66272	*J chem Soc (9)	1 870	12513	7
6	6	62041	J chem Physics	2 918	10462	9
7	8	51491	Biochim biophys Acta	3 120	14129	5
8	7	47505	Science	5 412	11781	8
9	9	46917	Proc natn Acad Sci USA	8 989	15317	4
10	11	37047	Lancet	6 677	10383	10
11	10	31563	Biochem J	3 627	4885	23
12	12	29275	Physical Rev Letters	5 059	10108	11
13	32	27080	Biochemistry	4 711	7325	17
14	25	26726	New Engl J Med	8 364	7385	15
15	22	24768	J clin Invest	6 992	5377	21
16	18	24209	J molec Biol	7 502	6129	18
17	41	23220	Biochem biophys Res Comm	3 744	8110	12
18	19	22520	J Physiol Lond	4 495	3160	46
19	33	22460	*Nuclear Physics (3)	2 514	7356	16
20	21	22245	*J Cell Biol (2)	6 770	3683	38
21	29	22201	Astrophys J	4 063	7451	14
22	14	21519	Am J Physiol	2 414	2412	59
23	27	20748	Brit med J	3 556	4829	24
24	36	20699	J expl Med	11 874	5557	19
25	15	20539	J org Chem	1 495	3526	40
26	16	19277	J appl Physics	1 558	3275	42
27	31	18375	J Bacteriology	2 727	3809	37
28	30	18190	Analytical Chem	3 291	4140	32
29	17	18171	Proc Soc expl Biol Med	1 471	2454	58
30	23	18086	J phys Chem	2 031	2768	54
31	26	17211	J Am med Ass	3 068	2982	49
32	20	17201	*Proc R Soc (3)	2 350	1114	135
33	13	16782	*C r Acad Sci (5)	0 529	4247	29
34	35	16509	Tetrahedron Letters	1 777	5004	22
35	38	15970	*Archs Biochem Biophys (2)	2 952	3050	48
36	53	15948	Endocrinology	4 337	4098	33
37	49	15826	J Immunology	5 112	4703	26
38	34	15666	*Physics Letters (2)	2 133	7672	13
39	39	15281	J geophys Res	2 536	3854	36
40	24	14706	*Chem Ber (2)	1 506	1353	104
41	37	14668	Ann N Y Acad Sci	1 181	1291	113
42	52	14461	Circulation	6 834	4025	34
43	50	14310	Inorg Chem	2 457	3589	39
44	45	13911	*Acta crystallographica (3)	1 361	2394	60
45	82	13847	*Eur J Biochem (2)	3 857	4595	27
46	47	13753	J Pharmacol expl Ther	3 576	2026	65
47	42	13072	Fedn Proc	0 489	4212	30
48	58	12544	Cancer Res	3 391	3164	45
49	69	11645	*J clin Endocr Metab (2)	5 170	3443	41
50	43	11459	*J Physics (7)	1 689	5450	20
51	28	11421	*Zh eksp teor Fiz (2)	1 565	1607	84
52	57	11371	Virology	3 752	2949	50
53	40	11294	*J Polym Sci (6)	0 964	1565	88
54	65	11127	Exp Cell Res	3 014	2788	53
55	48	10756	*Angew Chem (2)	4 140	2666	56
56	67	10231	Ann internal Med	4 828	2187	63
57	355	10227	Brain Res	3 104	4522	28

58	87	10206	Analytical Biochem	2 379	2184	64
59	46	9824	*Dokl Akad Nauk SSSR (7)	0 339	1681	81
60	62	9779	Am J Med	4 411	1535	90
61	76	9678	J natn Cancer Inst	3 289	2858	52
62	95	9497	Cancer	2 361	2056	66
63	59	9142	Can J Chem	1 396	1793	73
64	707	9094	FEBS Letters	3 049	4815	25
65	74	9082	Circulation Res	4 922	1698	79
66	108	9026	*Physica Status Sol (3)	1 476	3201	44
67	64	8903	Tetrahedron	1 576	1913	69
68	77	8890	Am J Obstet Gynec	2 100	2236	62
69	78	8835	Plant Physiol	2 580	1935	68
70	54	8803	*Acta chem scand (3)	1 042	1192	124
71	63	8798	J Lab clin Med	2 802	1132	131
72	113	8693	Gastroenterology	5 394	2260	61
73	107	8625	Appl Physics Letters	3 220	3246	43
74	70	8619	J appl Physiol	1 780	1184	125
75	481	8478	Applied Physics Letters	2 403	4205	31
76	141	8241	J organomet. Chem	2 392	3891	35
77	56	8183	Bull Soc chim. France	1 001	1492	96
78	81	7941	Bull chem Soc Japan	0 932	1859	72
79	132	7928	J Chromatography	2 173	2886	51
80	71	7922	Acta physiol scand	2 204	919	170
81	72	7914	J. phys Soc Japan	1 132	1500	95
82	61	7860	*Z Naturforschung (3)	1 070	1503	94
83	192	7794	J Neurochem	3 535	2464	57
84	106	7656	*Br J Pharmacol	3 516	1751	77
85	80	7459	Ann Surgery	2 129	1060	140
86	113	7335	*Cell Tissue Res (2)	1 961	1761	75
87	122	7183	J Pediatrics	2 600	1890	70
88	84	7120	Blood	4 319	1529	91
89	60	7117	Helv chim Acta	1 649	1034	144
90	68	7063	Philosophical Mag	1 836	876	178
91	147	7007	Biochem. Pharmacol	2 023	1689	80
92	100	6951	Pediatrics	2 502	1346	105
93	120	6811	Am J. Cardiol	3 704	1889	71
94	276	6788	J Virology	4 864	3142	47
95	149	6770	*J. Bone Jt Surg (3)	1 358	729	234
96	73	6662	Z Physik	1 340	864	182
97	112	6600	Experientia	0 883	1647	83
98	88	6539	J gen Physiol	4 308	741	229
99	51	6362	*Fizika tverd Tela (2)	0 762	1388	102
100	129	6307	Radiology	1 198	1320	107
101	66	6177	Annln Chemie (J Liebig)	1 024	432	379
102	89	6066	*Archs internal Med (2)	2 202	946	163
103	90	5994	Am Heart J	1 791	840	188
104	86	5885	J opt Soc Am	2 016	905	173
105	94	5849	*J Physics Chem Solids (2)	1 394	715	239
106	99	5761	J inorg nucl Chem	0 962	1149	128
107	156	5743	J Endocrinology	2 919	1757	76
108	217	5683	*J Pharmaceut Sci (3)	1 622	1549	92
109	92	5679	J gen Microbiol	2 160	1136	129
110	115	5675	Surgery	1 559	842	187
111	378	5573	Solid St Comm	1 945	2768	55
112	170	5557	Clin chim Acta	1 669	1587	86
113	150	5556	J Neurophysiology	4 537	676	249
114	98	5501	Methods Enzymology	1 765	547	311
115	136	5491	Archs Surgery	1 462	915	171
116	101	5486	Surgery Gynec Obstet	1 332	750	226
117	109	5478	J Electrochem Soc	1 053	1098	136
118	55	5474	*Nuovo Cimento (3)	0 994	999	155
119	123	5428	J acoust Soc Am	1 142	830	195
120	96	5388	Am J Pathol	2 807	856	184
121	91	5388	J expl Psychol	1 027	750	226
122	126	5363	*Spectrochim Acta (3)	1 487	840	188
123	83	5326	Genetics	2 835	995	157
124	158	5197	J Ultrastruct Res	2 709	837	190
125	103	5186	Revs mod Physics	21 500	731	231
126	121	5167	J Histochem Cytochem	4 005	757	224
127	102	5138	Anat Rec	2 884	649	265
128	235	5092	*Zh obshch Khim (2)	0 808	1050	142
129	192	5063	Immunology	2 816	1118	132
130	125	5053	J Nutrition	1 845	740	230
131	117	5038	Am J Roentg Rad Ther	1 008	634	272
132	166	5033	J Lipid Res	3 525	719	238
133	134	5031	J Urology	0 721	776	216
134	194	5000	Life Sciences	2 062	1200	121
135	177	4909	Acta endocrinologica	2 461	1383	103
136	267	4861	J infect Dis	3 040	1669	82
137	75	4847	Phytopathology	1 155	789	210
138	111	4822	Physics Fluids	1 188	972	159
139	116	4801	Rev scient Instrum	1 018	1001	153

140	160	4767	J Biochem Japan	1 715	1079	138	282	589	2831	Solar Physics 1967	1 929	1059	141
141	184	4707	Nucl Instrum Meth	1 050	1420	100	291	191	2725	NS Archs Pharmacol 1972	2 792	1033	145
142	127	4704	Z anorg allg Chem	1 019	593	286	230	319	3403	Annu Rev Biochem 1932	19 358	1026	147
143	159	4697	J comp Neurol	3 725	771	219	267	190	2946	Archs gen Psychiatry 1960	2 475	1022	150
144	105	4656	Can J Physics	1 038	774	218	237	500	3279	Psychopharmacologia 1959	2 347	1002	152
145	168	4655	Lab Investigation	2 940	932	166	352	776	2326	*Zh analyt Khim 1946 (2)	1 060	996	156
146	133	4604	Hoppe-Seylers Z physiol Chem	2 291	1031	146	370	416	2171	IEEE J. Quantum Electronics 1965	3 567	988	158
147	211	4603	Applied Optics	1 832	1539	89	232	327	3373	Biopolymers 1963	2 492	972	159
148	370	4600	Surface Science	3 340	1787	74	459	—	1592	Transplantation Revs 1969	25 579	972	159
149	224	4511	*Comp Biochem Physiol (3)	1 014	1250	116	256	326	3074	Chromosoma 1939	3 875	961	162
150	247	4480	Applied Microbiology	1 292	1196	122	356	—	2265	Metallurg Trans AIME 1970	1 054	939	164
151	155	4479	Am J clin Pathol.	1 348	663	255	434	—	1707	*Lettere Nuovo Cimento 1969 (2)	0 755	929	167
152	182	4462	Am J Surg	1 183	731	231	357	627	2258	J gen Virology 1967	2 501	928	168
153	220	4453	Molecular Physics	2 334	1258	115	468	—	1553	Optics Communications 1969	1 551	920	169
154	442	4451	*J comp. Physiol (2)	2 782	893	175	311	345	2551	*Chest 1970 (2)	1 253	916	172
155	137	4416	Am J Dis Child	1 495	809	202	323	505	2422	Mutation Res 1964	2 365	894	174
156	162	4393	*Archs Dermatology (3)	1 784	835	192	289	533	2738	Accus chem Res 1968	7 403	881	177
157	262	4369	Phytochemistry	1 103	1568	87	300	394	2630	*Agric biol Chem Tokyo 1961 (2)	0 982	867	179
158	110	4356	Acta Metallurgica	1 705	583	291	305	470	2559	Carbohydrate Res 1965	1 312	867	179
159	93	4353	*J comp physiol Psychol (2)	1 230	663	256	420	NA	1771	J Vacuum Sci Technol 1964	1 472	867	179
160	140	4348	Cold Spring Harb Symp	2 443	623	278	371	539	2159	*J chromatogr Sci 1969 (2)	3 196	847	186
161	159	4347	Ann Physics	2 128	598	284	338	512	2387	Earth planetary Sci Letters 1966	1 802	827	196
162	214	4308	Planta	2 589	1261	114	227	268	3423	Br J Haematol 1955	2 711	824	197
163	135	4303	Archs Pathology	1 521	508	332	321	530	2449	Clin Pharmacol Therap 1970	3 423	818	199
164	85	4277	*Proc IEEE (2)	2 013	781	215	253	277	3114	Obstet Gynecol 1953	1 367	816	200
165	147	4253	Pflugers Arch /Eur J Physiol	1 810	856	184	306	317	2557	Steroids 1963	3 189	810	201
166	238	4208	*J. Pharmacy Pharmacol (2)	3 140	1118	132	548	—	1281	J magn Resonance 1969	2 082	808	203
167	443	4180	*Zh neorg. Khim (2)	0 523	823	198	274	248	2893	Med J Australia 1914	0 725	805	204
168	199	4116	J Anim Sci	1 311	1000	154	304	389	2600	Izv Akad Nauk SSSR Khim 1936	0 540	802	205
169	153	4104	Chem Revs	11 154	580	293	220	361	3530	Expl Neurology 1959	1 827	793	207
170	161	4093	J thorac cardiovasc Surg	1 480	836	191	427	552	1740	J Crystal Growth 1967	2 503	791	209
171	180	4072	*J cell Physiol (2)	3 737	710	240	286	381	2767	*J Obst Gyn Br Comm 1961 (2)	1 922	786	211
172	286	4068	J Reprod Fert	2 357	1414	101	494	627	1453	Icarus 1962	3 489	785	212
173	274	4054	*Transplantation (2)	2 250	1134	130	277	343	2885	J Catalysis 1962	1 603	784	213
174	558	4049	Clin expl Immunol	4 423	1601	85	428	608	1728	*Pediatric Res 1967 (2)	4 399	783	214
175	176	4040	Coll Czech chem Comm	0 791	831	194	384	941	2035	Macromolecules 1968	2 276	776	216
176	169	4031	*Am Res rev Dis (3)	1 630	937	165	246	207	3155	Anesthesiology 1940	2 024	771	219
177	189	4023	Geochim cosmochim Acta	4 056	1160	127	241	293	3186	J agric Food Chem 1953	1 195	771	219
178	271	4005	Analytica chim acta	2 093	1312	110	257	311	3069	*J Atmosph Sci 1962 (2)	2 051	769	222
179	157	4003	*Deut med Wschr (2)	1 022	1025	149	390	374	1990	*Fiz Tekh Poluprovodn 1967 (2)	0 680	762	223
180	148	3996	Physiol Revs	13 861	499	334	357	736	2261	*Zh org Khim 1965 (2)	0 643	757	224
181	138	3993	Acta med scand	1 124	508	331	361	399	2222	Talanta 1958	1 787	731	231
182	195	3952	Diabetes	3 941	863	183	267	277	2949	*Can J Botany 1951 (2)	1 069	729	234
183	97	3932	*Zh fiz Khim (2)	0 331	646	266	251	255	3130	Archs Dis Childhood 1926	1 901	728	236
184	194	3906	Geol Soc Am Bull	1 674	1026	147	311	320	2547	Br J Cancer 1947	3 232	724	237
185	364	3899	Astronomy Astrophys	2 267	2018	67	233	164	3333	Makromolek Chemie 1945	1 088	704	241
186	172	3897	J Dairy Sci	0 273	569	300	425	—	1755	Org Mass Spectrometry 1968	1 088	704	241
187	218	3892	Neurology	2 181	796	206	259	235	3038	Br Heart J 1939	1 631	698	243
188	503	3874	*Int J Cancer (2)	4 928	1508	93	270	266	2927	*Nouv Presse Med 1972 (2)	0 612	696	244
189	367	3869	Clinical Chem	3 195	1460	97	387	576	2004	Toxicol appl Pharmacol 1959	1 672	689	245
190	171	3864	Am J Ophthalmol	1 389	792	208	349	334	2332	*Archs Microbiology 1974 (2)	1 468	684	246
191	178	3858	Mon Not R astr. Soc	1 421	1003	151	281	282	2845	Metabolism 1952	2 387	678	247
192	165	3857	Archs Ophthalmology	1 293	561	302	828	—	717	Kidney International 1972	3 740	677	248
193	154	3852	J Fluid Mech	1 254	617	280	326	639	2433	Expl Brain Res 1965	3 596	676	249
194	146	3827	*Ber Bunsenges	1 382	532	319	228	259	3414	J mol Spectroscopy 1957	1 744	675	251
195	160	3820	J math Physics	1 046	632	274	371	587	2147	Vision Research 1961	1 800	675	251
196	339	3777	*J mednl Chem (2)	1 444	1196	123	353	297	2321	Planetary Space Sci 1959	1 645	671	253
197	369	3726	Gut	3 336	1081	137	293	183	2696	Can J Biochem 1964	1 671	670	254
198	130	3710	Am J Botany	1 378	357	441	302	472	2621	Molec Pharmacol 1965	3 785	670	254
199	232	3701	J Neurosurgery	1 252	636	271	238	228	3259	J clin Pathol 1947	1 550	662	258
200	204	3699	Scand J clin Lab Invest	1 917	644	268	269	308	2931	J. Insect Physiol 1957	1 505	662	258
201	249	3673	*Archs Neurol. (2)	2 217	745	228	263	308	2987	Am J clin Nutrition 1954	1 714	658	260
202	599	3647	*Eur J Pharmacol (2)	2 537	1205	120	226	185	3453	Austral J Chem 1953	1 006	658	260
203	339	3633	Developmental Biol	3 384	1242	117	234	281	3321	*J Cell Sci 1966 (2)	2 973	657	262
204	196	3561	Arzneimittel-Forschung	0 876	833	193	317	NA	2505	J Fish Res Board Can 1938	1 053	656	263
205	202	3598	*Clin Sci mol Med (2)	2 474	762	223	294	230	2669	Bacteriol Revs 1937	16 795	655	264

b

284	—	2809	Cellular Immunol 1970	4 848	1721	78
244	174	3164	Bull Am. phys. Soc 1925	0 347	1459	98
376	—	2094	Eur J Immunol 1971	4 852	1441	99
348	—	2357	Infect Immunity 1970	2 032	1335	106
272	295	2919	*Clin Res 1958 (2)	0 198	1316	108
307	—	2556	Transplantation Proc. 1969	2 709	1314	109
487	—	1470	Prostaglandins 1972	5 247	1296	111
281	448	2850	*Molecular gen Genetics 1967 (2)	2 699	1293	112
242	370	3182	*J electroanal. Chem 1967 (2)	1 567	1222	118
239	744	3238	Physiol Behavior 1966	1 678	1171	126
335	—	2406	Antimicr. Ag Chemother. 1972	2 564	1118	132
323	507	2447	J nucl. Med 1960	3 040	1061	139

Perhaps the point to be stressed in presenting these data is the bibliographic law of concentration⁶. When the *SCI* was first reviewed in *Nature* more than a decade ago⁷, the scope of its journal coverage was called into question. I believe time has shown beyond doubt that the important literature of science is encompassed by fewer than 1,000 journals. And even fewer account for the truly significant. Of some 45,000 serials of all kinds received by the British Lending Library, two-thirds are rarely, if ever, subject of request. A small core of about 5,000 accounts for almost 80% of all requests⁸.

Fig. 2a, High-impact journals in 1974 (excluding review journals). Journals are listed in descending numerical order of 1974 impact factor. b, High-impact review journals. A: rank in terms of 1974 impact; B: 1974 impact; C: 1969 impact; D: total 1974 citations of 1972 and 1973 articles; E: total number of 1972 and 1973 articles.

a	A	B	C	D	E	81	3 137	2 593	Am J human Genetics	436	139
						82	3 135	1 981	Am Naturalist	326	104
1	11 874	8 307	J expl Med	5557	468	83	3 120	3 102	Biochim biophys Acta	14129	4529
2	8 989	8 566	Proc natn Acad Sci USA	15317	1704	84	3 104	3 486	Brain Res	4522	1457
3	8 364	2 359	New Engl J Med	7385	883	85	3 068	1 050	J Am med Assoc	2982	972
4	7 502	8 811	J molec Biol.	6129	817	86	3 049	NA	FEBS Letters	4815	1579
5	6 992	3 362	J clin Invest	5377	769	87	3 048	—	Differentiation	64	21
6	6 834	1 214	Circulation	4025	589	88	3 040	1 000	J infect Dis	1669	549
7	6 770	3 386	J Cell Biol	3683	544	88	3 040	0 505	J nuclear Med	1061	349
8	6 677	1 485	Lancet	10383	1555	90	3 016	—	Cognitive Psychology	190	63
9	5 843	6 059	J biol Chem	13685	2342	91	3 014	2 241	Expl Cell Res	2788	925
10	5 412	2 993	Science	11781	2177	92	2 973	4 918	J Cell Science	657	221
11	5 394	1 147	Gastroenterology	2260	419	93	2 967	3 230	Arch Biochem. Biophys	3050	1028
12	5 247	—	Prostaglandins	1296	247	94	2 940	2 008	Lab Investigation	932	317
13	5 170	3 868	J. clin Endocr Metab	3443	666	95	2 920	—	Bioinorganic Chem	73	25
14	5 112	4 121	J Immunology	4703	920	96	2 919	2 021	J Endocrinology	1757	602
15	5 059	4 911	Physical Rev Letters	10108	1998	97	2 918	3 128	J chem Physics	10462	3585
16	4 957	—	Scand J Immunology	570	115	98	2 916	—	Biol Reproduction	592	203
17	4 928	2 553	Int J Cancer	1508	306	99	2 884	0 409	Anat Rec	649	225
18	4 922	1 750	Circulation Res	1698	345	100	2 864	1 337	J Neuropathol expl Neurol	232	81
19	4 864	5 269	J Virology	3142	646	101	2 846	4 057	Q J Med	222	78
20	4 852	—	Eur J Immunology	1441	297	102	2 835	1 815	Genetics	995	351
21	4 848	—	Cell Immunology	1721	355	103	2 823	—	J immunol Meth	223	79
22	4 828	1 679	Ann internal Med	2187	453	104	2 816	3 859	Immunology	1118	397
23	4 711	5 694	Biochemistry	7325	1555	105	2 807	1 814	Am J Pathol	856	305
24	4 537	4 435	J. Neurophysiology	676	149	106	2 802	1 702	J Lab clin Med	1132	404
25	4 495	2 432	J. Physiol Lond	3166	703	107	2 792	1 266	NS Arch Pharmacol	1033	370
26	4 423	3 363	Clin expl Immunology	1601	362	108	2 782	1 638	J comp Physiol	893	321
27	4 411	4 516	Am J. Med	1535	348	109	2 727	3 341	J Bacteriology	3809	1397
28	4 399	0 680	Pediatric Res	783	178	110	2 711	2 658	Br J Hematol	824	304
29	4 383	5 164	J Am chem Soc	17088	3899	111	2 709	3 012	J Ultrastruct Res	837	309
30	4 340	NA	Seminars Hematology	204	47	111	2 709	—	Transplantation Proc	1314	144
31	4 337	2 906	Endocrinology	4098	945	113	2 704	3 596	Physical Rev	19174	7092
32	4 319	2 219	Blood	1529	354	114	2 699	2 880	Molecular gen Genetics	1293	479
33	4 380	2 968	J gen Physiol	741	172	115	2 600	—	Intervirology	91	35
34	4 140	2 925	Angew. Chemie	2666	644	115	2 600	1 374	J Pediatrics	1890	727
35	4 063	4 661	Astrophys. J	7451	1834	117	2 589	2 944	Planta	1261	487
36	4 060	0 672	Arthritis Rheumatism	613	151	118	2 580	1 573	Plant Physiol	1935	750
37	4 056	2 725	Geochim cosmochim Acta	1160	286	119	2 564	—	Antimicrob Agents Chemother	1118	436
38	4 006	2 342	Nature	18924	4724	120	2 545	0 916	Biophysical J	514	202
39	4 005	2 287	J Histochem Cytochem	757	189	120	2 545	—	Eur J clin Invest	280	110
40	3 967	2 090	Cytogenet Cell Genetics	357	90	120	2 545	—	J molecular Evolution	112	44
41	3 941	2 039	Diabetes	863	219	123	2 537	3 661	Eur J Pharmacol	1205	475
42	3 875	2 767	Chromosoma	961	248	124	2 536	3 385	J geophys Res	3854	1520
43	3 875	3 976	Eur J Biochem	4595	1186	125	2 528	—	Radiation Effects	493	195
44	3 796	—	Tissue Antigens	429	113	125	2 528	2 836	Nuclear Physics	7356	2910
45	3 785	3 916	Molecular Pharmacol	670	177	127	2 513	—	Thrombosis Res	392	156
46	3 752	4 486	Virology	2949	786	128	2 512	4 965	J Petrology	103	41
47	3 744	4 292	Biochem biophys Res Comm	8110	2166	129	2 503	2 277	J Crystal Growth	791	316
48	3 740	—	Kidney International	677	181	130	2 502	1 495	Pediatrics	1346	538
49	3 737	3 488	J cell Physiol	710	190	131	2 501	2 894	J gen Virology	928	371
50	3 726	—	Clin Immunol Immunopathol	231	62	132	2 492	2 791	Biopolymers	972	390
51	3 725	2 335	J comp Neurology	771	207	133	2 484	3 232	Immunochimistry	611	246
52	3 704	2 170	Am J Cardiology	1889	510	134	2 481	NA	In Vitro	258	104
53	3 627	3 060	Biochem J	4885	1347	135	2 475	1 409	Archs gen Psychiatry	1022	413
54	3 596	4 783	Expl Brain Res	676	188	136	2 474	2 732	Clin Sci mol Med	762	223
55	3 576	3 568	J Pharmacol expl Ther	2060	576	137	2 467	4 307	Mon Not R astr Soc	1036	420
56	3 567	1 307	IEEE J Quantum Electronics	988	277	138	2 464	—	Expl Hematology	69	28
57	3 556	0 677	Br med J	4829	1358	139	2 461	1 316	Acta endocrinologica	1383	562
58	3 535	2 884	J Neurochemistry	2464	697	140	2 457	3 188	Inorg Chemistry	3589	1461
59	3 525	3 876	J Lipid Res	719	204	141	2 447	2 873	Neuroendocrinology	438	179
60	3 516	2 658	Br J Pharmacol	1751	498	142	2 443	5 463	Cold Spring Harbor Symp	623	255
61	3 489	1 697	Icarus	785	225	143	2 441	1 685	Neuropharmacology	554	227
62	3 441	3 401	Br med Bull	320	93	144	2 414	3 115	Am J Physiology	2412	999
63	3 423	1 657	Clin Pharmacol Ther	818	239	145	2 413	—	Hormones Behavior	193	80
64	3 391	2 879	Cancer Res	3164	933	146	2 403	2 477	Chem Physics Letters	4205	1750
65	3 384	3 729	Developmental Biol	1242	367	147	2 392	3 497	J organomet Chem	3891	1627
66	3 340	2 629	Surface Science	1787	535	148	2 387	2 088	Metabolism	678	284
67	3 336	1 174	Gut	1081	324	149	2 379	3 330	Analyt. Biochem	2184	918
68	3 291	1 605	Analyt Chem	4140	1258	150	2 375	0 326	Am Zoologist	342	144
69	3 289	4 009	J natn Cancer Inst	2858	869	151	2 365	2 497	Mutation Res	894	378
70	3 266	—	J Membrane Biol	578	177	152	2 361	2 064	Cancer	2056	871
71	3 232	1 670	Br J Cancer	724	224	153	2 357	2 014	J Reprod Fert	1414	600
72	3 220	3 545	Applied Physics Letters	3246	1008	154	2 355	NA	J psychiat Res	73	31
73	3 215	NA	J Allergy clin Immunol	463	144	155	2 350	3 085	Proc R Soc Lond	1114	474

156	2 349	3 662	Psychol Bull	444	189	27	6 433	9 600	Adv Enzymology	193	30
157	2 347	2 380	Psychopharmacologia	1002	427	28	6 357	3 384	Erg physiol biol Chem exp Pharm	89	14
158	2 337	—	Drug Metab Dispositio	236	101	29	6 133	NA	Adv organomet Chem	92	15
159	2 334	2 173	Molecular Physics	1258	539	30	6 083	18 000	Prog phys org Chem	73	12
160	2 311	2 561	Faraday Disc chem Soc	208	90	31	6 000	NA	Topics Stereochem	24	4
161	2 297	1 374	J. Verbal Learning Verbal Behav	395	172	32	5 733	—	Annu Rev Biophys Bioenegng	172	30
162	2 291	1 636	Hoppe-Seylers Z. physiol Chem	1031	450	33	5 689	—	Chem Soc. Revs	256	45
163	2 286	—	Organic Mass Spectrometry	704	308	34	5 500	NA	Int Rev Cytology	209	38
164	2 279	—	J Neurocytology	139	61	35	5 444	—	Adv cell molec Biol	49	9
165	2 276	2 529	Macromolecules	776	341	36	5 214	—	Q Rev Biophysics	73	14
166	2 268	2 061	Photochem Photobiol	542	239	37	5 045	NA	Adv Quantum Chem	111	22
167	2 267	0 987	Astronomy Astrophysics	2018	890	38	5 000	NA	Adv Colloid Interface Sci	25	5
167	2 267	—	J Steroid Biochem	390	172	38	5 000	NA	Electroanalyst Chem	15	3
169	2 262	0 842	Invest Ophthalmology	579	256	38	5 000	3 647	Vitamins Hormones	55	11
170	2 250	3 164	Transplantation	1134	504	41	4 923	—	Adv cyclic Nucleotide Res	256	52
171	2 237	0 869	Gen comp Endocrinol	633	283	42	4 775	6 545	Annu Rev Microbiol	191	40
172	2 234	—	Cell Tissue Kinetics	239	107	43	4 690	5 176	Biol Revs Cambridge Phil Soc	136	29
173	2 217	1 449	Archs Neurology	745	336	44	4 500	16 285	Solid St. Physics	45	10
174	2 205	1 514	Brain	291	132	45	4 375	NA	Int. Rev Neurobiol	35	8
175	2 204	2 479	Acta physiol scand	919	417	46	4 339	4 685	Rev Geophys Space Physics	269	62
176	2 200	1 769	Archs internal Med	946	430	47	4 300	—	Adv Human Genetics	43	10
177	2 199	NA	Analytical Letters	497	226	48	4 188	5 000	Medicine	268	64
178	2 193	NA	Physics Today	182	83	49	4 176	NA	Adv microb Physiol	71	17
179	2 181	0 868	Neurology	796	365	50	4 156	4 433	Psychol. Rev	320	77
180	2 173	1 271	J Chromatography	2886	1328	51	4 000	NA	Adv Lipid Res	52	13
181	2 160	2 127	J gen Microbiology	1136	526	52	3 783	5 629	Annu Rev nucl Sci	87	23
182	2 151	—	J non-crystalline Solids	628	292	53	3 750	4 695	Coordination Chem Revs	255	68
183	2 147	2 876	Diabetologia	307	143	53	3 750	NA	Prog med Virol	60	16
184	2 134	2 359	Physics Letters	7672	3595	55	3 500	3 555	Annu Rev phys Chem	133	38
185	2 129	1 613	Ann Surgery	1060	496	55	3 500	NA	Prog med Genetics	49	14
186	2 128	3 089	Ann Physics	598	281	57	3 462	NA	Prog Surf Membrane Sci	45	13
187	2 100	1 207	Am J Obstet Gynecol	2236	1065	58	3 412	7 333	Adv Virus Res	58	17
188	2 096	NA	Eur J clin Pharmacol	262	125	59	3 000	NA	Adv metab Disorders	21	7
189	2 093	0 965	Analytica chim Acta	1312	627	59	3 000	3 818	Botanical Rev	66	21
190	2 090	2 027	Eur J Cancer	466	223	59	3 000	—	Drug Metab Revs	42	14
191	2 083	1 787	Acta mathematica	75	36	59	3 000	NA	Essays Biochem	27	9
192	2 082	—	J magnetic Resonance	808	388	59	3 000	NA	Prog Materials Sci	15	5
193	2 073	2 252	Expl Eye Res	537	259	64	2 923	NA	Catalysis Revs	76	26
194	2 071	—	Cell Differentiation	145	70	65	2 909	4 500	Prog cardiovasc Dis	160	55
195	2 062	1 839	Life Sciences	1200	582	66	2 900	NA	Int Rev expl Pathol	29	10
196	2 056	—	Contraception	368	179	67	2 844	8 296	Rep Prog Physics	128	45
197	2 054	1 643	Int J Radiation Biol	456	222	68	2 746	4 235	Annu Rev Medicine	173	63
198	2 051	2 016	J Atmospheric Sci	769	375	69	2 456	4 000	Adv Enzyme Regulation	106	43
199	2 041	1 195	J Antibiotics Tokyo	445	218	70	2 462	0 176	Q Rev Biology	64	26
200	2 032	—	Infection Immunity	1335	657	71	2 273	5 600	Adv Carbohydr Chem Biochem	25	11
201	2 031	2 329	J phys Chem	2768	1363	72	2 250	2 888	Harvey Lectures	36	16
202	2 024	2 040	Aesthesiology	771	381	73	2 200	NA	Adv clin Chem	22	10
203	2 023	1 888	Biochem Pharmacol	1689	835	74	2 188	NA	Adv Pharmacol	35	16
204	2 022	1 855	Theor chim Acta	645	319	75	2 086	NA	Annu Rev Psychol	73	35
205	2 016	0 904	J opt Soc Am	905	449	76	2 079	5 485	Annu Rev Entomology	79	38
206	2 013	1 372	Proc Instrn electl electr Engrs	781	388	77	2 071	NA	Applied Spectrosc Rev	29	14
						78	2 047	4 914	Annu Rev Phytopathol	88	43

b

1	25 579	—	Transplantation Revs	972	38
2	22 643	9 600	Adv Immunology	317	14
3	21 500	4 317	Revs mod Physics	318	34
4	19 358	17 584	Annu Rev Biochem	1026	53
5	16 795	20 615	Bacteriol Revs	655	39
6	15 778	NA	Curr Topics Microbiol	142	9
7	13 861	17 333	Physiol Revs	499	36
8	12 545	13 428	Progr Allergy	138	11
9	11 613	8 592	Rec Progr Hormone Res	360	31
10	11 154	8 160	Chem. Revs	580	52
11	9 700	8 888	Adv inorganic Chem Radiochem	97	10
12	9 577	22 400	Pharmacol Revs	498	52
13	9 200	3 259	Adv chem Physics	92	10
14	8 379	7 743	Annu Rev Astr Astrophys	243	29
15	7 875	9 176	Prog Biophys molec Biol	189	24
16	7 833	—	Curr Topics cell Regulation	94	12
17	7 765	20 200	Prog nucleic Acid Res	132	17
18	7 403	17 083	Accts chem Res	881	119
19	7 375	3 688	Adv Physics	177	24
20	7 316	7 047	Annu Rev Plant Physiol	278	38
21	7 143	NA	Curr Topics dev Biol	50	7
22	7 000	NA	Annu Rev Pharmacol	329	47
23	6 963	NA	Adv Cancer Res	188	27
24	6 679	NA	Annu Rev Genetics	187	28
25	6 636	23 000	Adv Protein Chem	73	11
26	6 581	4 216	Annu Rev Physiol	204	31

In using the data presented here, one should be aware that we revised our definition of "source items" used to calculate impacts. In 1969 we included as source items much material (editorials, non-scientific and non-technical correspondence, news notes, and so on) that does not by its very nature invite citation in scientific and technical reports. This policy worked to the disadvantage of some major journals. Our redefinition accounts in part for the changed impact in 1974 of journals like *Nature*, *Science*, *Lancet*, *Journal of the American Medical Association*, and *British Medical Journal*.

What is the significance of journal impact? By demonstrating that only 150 journals have impacts greater than 3, I believe we have established the futility of discussions based on the assumption that the average library must acquire and store thousands of journals. Since the average impact in 1974 was 1.015, any of the journals listed in the figures is likely to be a good candidate for selection.

Fig 2(b) shows clearly the importance of review journals, confirming our earlier studies. Their extraordinary impact, along with a surge in the number of review-type articles and publications, led to ISI's decision to publish *Index to Scientific Reviews*.

Clearly, a large part of the scientific record is of low impact. Only careful study can show whether this fact

Fig. 3 Significant journals in three scientific specialities. Each list gives journal, (A) total 1974 citations, (B) impact factor, (C) total 1974 citations of 1972 and 1973 articles, (D) number of 1972 and 1973 articles. Journals are listed in alphabetical order. The botany journals include all with more than 600 citations or an impact greater than 1. The astronomy/astrophysics journals include all with more than 400 citations or an impact greater than 0.8. The mathematics journals include all with more than 500 citations or an impact greater than 0.5.

BOTANY

Journal	A	B	C	D
Am J Botany	3710	1 378	357	127
Ann Botany	1674	1 069	232	130
Annu Rev Phytopathol	566	2 047	88	21
Annu Rev Plant Physiol	1760	7 316	278	19
Bot Review	585	3 000	66	5
Can J Botany	2897	1 069	729	343
J expl Botany	1762	1 506	369	120
J Phycology	653	1 409	193	74
Mycologia	1143	0 607	176	128
New Phytologist	1405	1 158	300	115
Physiol Plant Pathol	206	1 152	114	49
Physiol Plantarum	2617	1 555	479	196
Physiol Veget	322	1 172	116	43
Phytochemistry	4369	1 103	1568	624
Phytopathology	4842	1 155	789	372
Plant Cell Physiol	1223	1 164	327	115
Plant Dis Reporter	1489	0 413	307	379
Plant Physiology	8835	2 580	1935	373
Planta	4308	2 589	1261	219
Trans Br Mycol Soc	947	0 610	186	171
Z Pflanzenphysiol	1008	1 340	351	180

ASTRONOMY/ASTROPHYSICS

Journal	A	B	C	D
Ann Geophysique Paris	588	0 786	110	28
Annu Rev Astron Astrophys	955	8 379	243	17
Astron Zh	738	0 435	171	194
Astronomical J	2383	1 953	545	182
Astronomy Astrophysics	3899	2 267	2018	497
Astrophys J	22201	4 063	7451	1040
Astrophys Letters	879	1 209	347	—
Astrophysics Space Sci	963	1 048	395	194
Earth planetary Sci. Letters	2387	1 802	827	189
EOS Trans Am geophys Union	625	12 967	389	28
Geochim cosmochim Acta	4023	4 056	1160	134
Icarus	1453	3 489	785	150
J atmosph Sci	2630	2 051	769	211
J atmosph terrest Physics	1886	1 322	509	210
J geophys Res	15281	2 536	3854	791
J Spacecraft Rockets	421	0 334	139	199
Mon Not R astron Soc	3858	2 467	1036	249
Planetary Space Sci	2321	1 645	671	155
Publ astron Soc Japan	360	0 874	83	44
Publ astron Soc Pacific	1191	1 081	308	161
Publ Dominion astrophys Observatory	136	1 250	10	2
Q J R astron Soc	128	0 923	48	20
Solar Physics	2831	1 929	1059	282
Revs Geophys Space Physics	872	4 339	269	40
Sov Astronomy/AJ	456	0 295	116	194
Space Sci Revs	637	1 718	177	34
Z Astrophysik	597	—	—	—

MATHEMATICS

Journal	A	B	C	D
Acta Math	675	2 083	75	18
Adv Math	137	0 647	44	50
Am J Math	1064	0 474	54	38
Ann Mathematics	1921	1 226	103	35
Bull Am math Soc	1281	0 516	221	241
Comm pure appl Math	750	0 598	49	25
C r Acad Sci A	845	0 210	360	688
Duke math. J	711	0 391	70	86
Indiana Univ math J	207	0 590	111	94
Inventiones math	383	0 808	105	67
J Algebra	834	0 775	248	213
J differential Equations	375	0 610	111	60
J math Anal Appl	871	0 393	190	235
J Math pure appl	201	0 879	29	27
Math Annln	1190	0 381	123	145

Math Computation	602	0 557	107	109
Math Z	1150	0 471	164	152
Michigan math J	275	0 482	40	38
Pacific J Math	1133	0 279	180	239
Phil Trans R Soc A	1765	1 016	188	43
Proc Am math Soc	1725	0 304	433	516
Proc Cambridge phil Soc	1348	0 397	91	103
Proc London math Soc	834	0 533	81	78
Q appl Math	538	0 505	49	43
SIAM J math Analysis	107	0 467	56	93
SIAM J num Analysis	333	0 662	100	89
Studia math	506	0 491	106	59
Studies appl Math	99	0 615	32	20
Trans Am math Soc	2622	0 488	371	340

supports or contradicts the idea that science is built on the accumulated results of average effort that prepare the way for breakthroughs¹⁰. In any event, the data seem to me to warrant an examination of the cost-effectiveness of the present publishing system. Journals devote to the mass of rarely cited papers the same resources as to the small part that citation analysis shows to be important. Less than 1% of all papers cited will be cited ten or more times in any annual *SCI*. Although more than 40 million references have been processed for the *SCI* during the past fifteen years, only 116,400 papers have been cited ten times or more in any one year.

One would hope that the availability of *Journal Citation Reports* will have a salutary effect on editorial complacency. A change in a journal's citation rate or impact rate is proper reason for editorial concern, admitting that factors beyond editorial control may be responsible. Thus, a drop in the impact of the leading Soviet journal of physics, *Zhurnal Eksperimentalnoi i Teoreticheskoi Fiziki*, or a rise in the impact of *Teploenergetika* (translated as *Thermal Engineering*) may reflect a shift in interest or emphasis of research worldwide. But a change in citation rate or impact rate may just as likely reflect a change in quality of output.

Journal citation analysis can be quite complex in some cases. The problem of Soviet publications is one such case. Apart from the usual bibliographic problems encountered, one must deal with the fact that most leading Soviet journals appear in two versions, Russian and English. *Fizika i Tekhnika Poluprovodnikov* appears in English as *Soviet Physics Semiconductors*. Clearly that is not a close translation of the title, much less a transliteration. Such bibliographic casualness about titles is bad enough, but there is worse. Most of the retitled translations appear about a year after the originals. This means, if one assumes that the translation is the major stimulant of subsequent citation in Western journals, that the citable life of the Soviet literature is unfairly shortened at the outset by an overlong gestation period. And the outset is important, for if an article is going to be cited, it is most likely to be cited during the first or second year after publication. In the case of Russian journals, citations contributed by translated versions are usually out of phase with those of the rest of the literature. To assure confusion worse confounded, some of the translated versions have volume and page numbers different from the Russian originals. In our tabulations for the *JCR*, we have as far as possible compensated for these annoying vagaries.

As the data show, new journals can achieve high impact quickly. Good examples are *Cellular Immunology* (first published 1970) and *Prostaglandins* (1972). Their total 1974 citation counts were 2,809 and 1,470 respectively; their impacts, 4.848 and 5.247. Among the newer journals the 'European' journals are especially notable in this respect. *FEBS Letters* (began 1968, impact 3.049); *European Journal of Biochemistry* (began 1967, impact 3.874); *European Journal of Immunology* (began 1971, impact 4.852). We must hope that internationalisation of journals will continue. I believe that Latin-American, Asian, and African

journals would do well to consolidate in like manner to produce fewer but larger journals. It is clear that a large journal, even if less than first class, is more difficult to ignore than a smaller journal with equal and perhaps greater impact.

In some cases, however, consolidation is inappropriate and may be detrimental. Take, for example, *Journal of the American Chemical Society (JACS)* and *Journal of the Chemical Society*. The *Journal of the Chemical Society* encompasses nine different subtitled journals. If one were to consolidate comparable journals of the American Chemical Society, their total citation count would be about 183,000, almost double the 98,995 of the *JACS*. The impact of this conglomerate would, however, be only 3.381 (respectable enough) rather than 4.383. Insistence by the Chemical Society upon corporate identity for its journals by means of an identical "main title" with repeatedly retitled sections is the source of bibliographic confusion, as well as of much tedious work in sorting out citation data. It seems to me that most commercial publishers would have refused to scrap a title as well-known as *Transactions of the Faraday Society*. In my opinion, the umbrella of a corporate main title for all a society's journals does little for their individual identities.

I have avoided commentary on the performance of specific

journals, preferring to use the space granted me here for data rather than comment and speculation. And I have published many such analyses, usually on a categorical basis in *Current Contents*. All of them have had the same purpose, and lead to the same general conclusion. Science needs objective criteria for measuring the performance of journals. Citation analysis seems to offer a sound beginning. Considering the paucity of management tools available to the average science librarian—general or specialist—and considering as well the often prejudicial role of individual scientists in journal selection (we all have our favourite journals), I feel that the *JCR* data can provide a more reliable basis for journal selection than any we have had until now.

- ¹ Garfield, E., *Science*, 178, 471–479 (1972).
- ² *Journal Citation Reports (JCR)* 1 *Journal Ranking Package*. 2. *Source Data Package*. 3. *Reference Data Package* (Institute for Scientific Information, Philadelphia, 1973).
- ³ Garfield, E., *Journal Citation Reports, a Bibliometric Analysis of References Processed for the 1974 Science Citation Index*, Science Citation Index 1975 Annual, 9 (Institute for Scientific Information, Philadelphia, 1976).
- ⁴ Garfield, E., *Current Contents*, 6, 5–7 (1976).
- ⁵ Garfield, E., *Journal Citation Reports*, Section 2, 2–4 (1976).
- ⁶ Garfield, E., *Current Contents*, 31, 5–6 (1971).
- ⁷ Cleverden, C. W., *Nature*, 203, 446 (1964).
- ⁸ Line, M. B., and Wood, D. N., *J. Documentation*, 3, 234–245 (1975).
- ⁹ *Index to Scientific Reviews 1974 Annual, an International Interdisciplinary Index to the Review Literature of Science, Medicine, Agriculture, Technology, and the Behavioral Sciences*, 2 vols (Institute for Scientific Information, Philadelphia, 1975).
- ¹⁰ Cole, J., and Cole, S., *Science*, 178, 368–374 (1971).

articles

A model for ocean-floor metamorphism, seismic layering and magnetism

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Ocean-floor metamorphism, which overprints the pseudo-stratigraphy of ophiolite complexes in southern Chile, is best explained by a combination of hydrothermal and contact metamorphism associated with igneous activity at a spreading centre. Such metamorphism may be more important than igneous processes in producing the observed seismic layering and magnetic properties of the ocean floor.

MODELS of accretionary processes of the oceanic lithosphere integrate marine geophysical data, results of oceanic dredging, and studies of the pseudo-stratigraphy and petrology of ophiolites, recognised as sections of oceanic crust^{1–3}. An important element in these models is the relative extent to which primary igneous processes or secondary metamorphism best explain the geophysical properties of the ocean crust. In this paper we describe the overprint of ocean-floor metamorphism on the pseudo-stratigraphy of ophiolite complexes in southern Chile. The metamorphism in these and other ophiolites, as well as in the ocean floor, is better explained by hydrothermal and contact metamorphism associated with igneous processes at a spreading centre than by burial metamorphism⁴. The effects of this special type of metamorphism, as discussed below, may be more important in producing the geophysical properties of the ocean crust than the igneous processes with which they are associated.

Ocean-floor metamorphism

The ophiolite complexes in southern Chile represent the floor of a Mesozoic marginal basin⁵. Their igneous pseudo-

stratigraphy, like that of many ophiolites elsewhere, consists of pillow lavas underlain by a sheeted dyke complex in turn intruded by chemically layered gabbroic plutons (Fig. 1). The complex sequence of continued intrusion and extrusion which leads to the formation of this pseudo-stratigraphy is described elsewhere^{6,7}.

The ophiolite stratigraphy is overprinted by at least two metamorphic episodes, the second of which is a regional event associated with regional deformation, which only affects the rocks surrounding the ophiolites⁸. The associated low-grade regional metamorphism has not significantly altered the ophiolites and its effects can be easily distinguished.

The earlier phase of metamorphism is not related to regional deformation and its effects are restricted to the ophiolites. From the top of the ophiolites, the intensity of metamorphism increases downwards from zeolite facies through greenschist to amphibolite facies within a short vertical sequence of 2–3 km (Fig. 1). Completely fresh pillow lavas at the top of the sequence have not been encountered. The maximum metamorphic grade is attained within the gabbro unit where the metagabbroic rocks can be classified as amphibolites. Below this zone the metamorphic effect dies off rapidly, leaving fresh gabbros. This boundary between fresh gabbro and amphibolite is extremely irregular, deeper penetration of the metamorphic effect being concentrated along fractures, veins, shear zones and dykes. Similar effects have been observed associated with zones of deformation in some of the Italian Apennine ophiolites⁹.

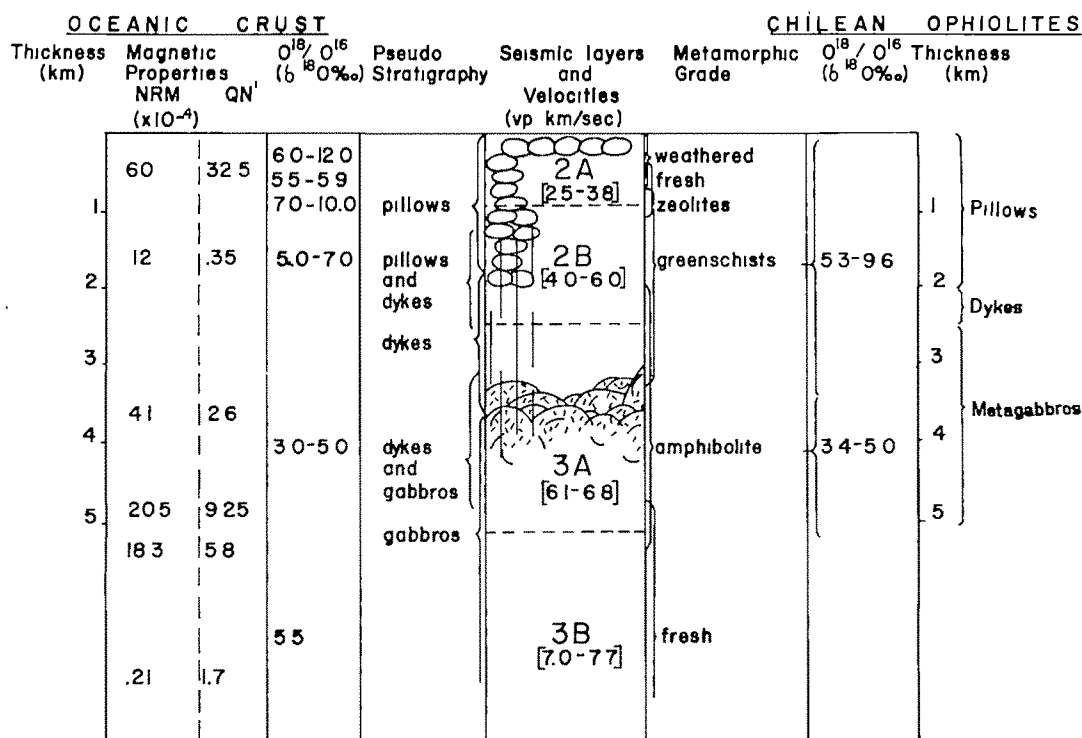


Fig. 1 A schematic column for Chilean ophiolites and a model of oceanic crust. The figure was constructed with preliminary oxygen isotope data for the Chilean ophiolites provided by J. Lawrence, and published seismic¹⁹, magnetic²¹, and oxygen isotope²⁰ data which have been correlated with metamorphic zones in the model of oceanic crust.

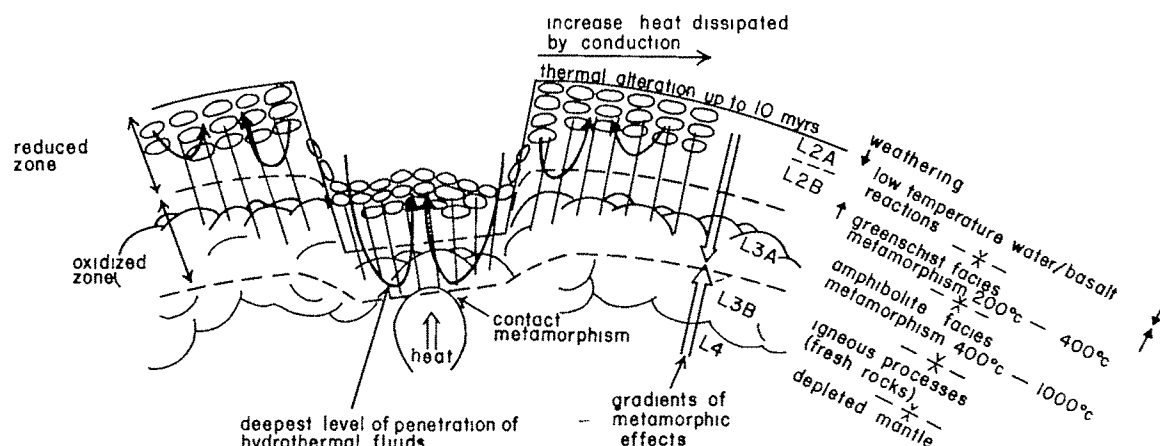
In detail, our petrographic data indicate that at the onset of the lower boundary of the amphibolite-facies metamorphism, olivine in olivine-pyroxene gabbros breaks down to skeletal magnetite and minor serpentine. Pyroxene develops thin rims of hornblende and plagioclase remains essentially unaltered. Higher in the section, pyroxene becomes uranitized and forms iron-rich hornblende. Skeletal networks of secondary magnetite form along fractures and cleavage planes of pyroxenes and amphiboles. Successively upwards, calcic plagioclase is replaced by clino-zoisite, epidote, chlorite, and reacts with silica to form myrmekites. Throughout these states of alteration, the metagabbros retain their original coarse ophitic igneous textures. The overprinted fine-grained metamorphic textures include dendrites and symplectites of epidote, chlorite, and, amphibole; myrmekites and granophyres of low Ca-plagioclase and quartz, and widmanstätten-like textures displayed by amphiboles and opaques. Grain boundaries are often ragged

and feathery. Such textures do not conform with normal textures associated with regional (dynamothermal) amphibolite-facies metamorphism.

Above this zone, metamorphic grade decreases to the greenschist facies. Textures conform with those observed in rocks from other ophiolite terrains and from oceanic dredge hauls. Original igneous textures are not always preserved, and mineralogically the metabasalts and metadolerites can be termed spilites¹².

We interpret the observed textures to be the result of metamorphism-metasomatism during which hot aqueous solutions incongruently dissolve and reprecipitate various elements. The breakdown of olivine and the high solubility of silica relative to iron at high temperatures results in the differential removal of Si with respect to Fe. Fe remains partially *in situ* in the form of magnetite, indicating an oxidising environment. The migration of fluids containing both dissolved Si and Fe, upwards along the decreasing

Fig. 2 Model of hydrothermal-contact metamorphism at a spreading centre, as outlined in the text. The figure shows the deep penetration of hydrothermal cells close to the ridge axis and their diminution away from the main zone of igneous activity and tectonism. The deepest level of penetration of the hydrothermal cells defines the depth to the bottom of layer 3A.



thermal gradient, results in the local precipitation of secondary magnetite as well as the formation of iron-rich hornblende from pyroxene. At successively higher levels, lower temperature decreases the solubility of Si, which instigates the reaction between silica and plagioclase to form myrmekites-dendrites and quartz. At still higher levels (dykes and pillow lavas) under lower prevailing temperatures, local metasomatism causes chemical and mineralogical changes termed spilitisation, which have been described previously⁹⁻¹². At this level sulphide mineralisation is common in ophiolites¹¹ and the environment seems to be reducing (Fig. 2).

A deuteric rather than hydrothermal process has been suggested as a possible cause for such a sequence of alterations^{11,14}. Conate fluids would be expected to be enriched at the top of magma chambers; however, the estimated weight percent of juvenile waters in oceanic tholeiites is low¹⁵. Hydrothermal circulation of seawater is a more likely explanation for the large scale (volume) alteration⁸. Preliminary data from the Chilean ophiolites (J. Lawrence, unpublished data) show a decrease in $\delta^{18}\text{O}$ from 9‰ in pillow lavas and pillow breccias, to 3.5‰ in the lowest metagabbros. Less altered underlying gabbros have values of 6.5‰, more representative of fresh oceanic gabbros and basalts (Fig. 1). Such a sequence from ^{18}O -enriched to ^{18}O -depleted rocks of basaltic compositions can be explained by re-equilibration, at successively higher temperatures, with a large reservoir of non-magmatic water.

Simple burial metamorphism is unlikely to be the process responsible for these features. Oceanic heat flow data indicate that the geothermal gradient needed to produce the observed metamorphic gradient would be encountered only in a narrow zone restricted to oceanic ridges¹⁶. Bonatti *et al.*⁴ drew identical conclusions from their study of metagabbros (amphibolites) dredged from the axial valley of part of the mid-Atlantic ridge, which their calculations showed had never been buried more than 1.5 km. They concluded that such a steep metamorphic gradient could be better explained by hydrothermal and contact metamorphism at the oceanic ridges, a process ultimately related to igneous intrusion beneath and volcanism within the median valley of such ridges. We agree with their conclusions and believe that such a model explains the steep metamorphic gradient in the upper section of our and other ophiolites (that is, the Troodos, Apennine, and Bay of Islands Complexes^{4,17}) as well as sections of the oceanic crust⁸. More significantly it can also explain the rapid termination of the metamorphic effects we observe within the gabbroic layer of the Chilean ophiolite complexes.

In simple terms, the model of Bonatti *et al.*⁴ involves a hydrothermal convective cell driven by the heat from igneous intrusions (Fig. 2). Seawater penetrates the oceanic layer along zones of high porosity and structural weakness, to a depth where it is driven back by heat from the intrusion of gabbro and dykes. Extensive hydrothermal systems within active spreading axes are supported by several lines of evidence. First, heat flow observed at spreading centres is lower than theoretical predictions and is best explained by a major component of convective in addition to conductive heat transfer¹⁸. Second, it has been demonstrated that such hydrothermal convective processes are the fundamental cause of metallogenic deposits forming at ridge crests¹⁹. $^{18}\text{O}/^{16}\text{O}$ studies indicate extensive interaction of dredged metabasalt and metagabbro with ocean water²⁰. Such hydrothermal systems have been confirmed by direct observation in Iceland where the spreading centre emerges²¹.

A number of factors indicate that the residence time of the metamorphosed gabbroic rocks within the zone of hydrothermal circulation is short, implying that deeper parts of this zone are narrowly restricted to the spreading

axis. The large surface areas of the myrmekite-dendrite textures indicate rapid growth under supersaturated conditions^{22,23}. Lack of recrystallisation and annealing textures indicates a rapid decrease in local temperature or fluid phase, or both and the absence of retrograde features substantiates this. In metagabbros dredged from the ocean floor, Muehlenbachs and Clayton²⁰ reported disequilibrium between $^{18}\text{O}/^{16}\text{O}$ ratios of coexisting plagioclase and pyroxene which they attributed to a kinetic effect whereby the pyroxene does not react as rapidly as the plagioclase. Such chemical disequilibrium, like textural disequilibrium, implies that the metagabbro had been removed rapidly from the zone of effective metamorphism. Two effects which may combine to restrict the width of the deep zone of hydrothermal alteration are (1) its dependence on intrusive and tectonic activity, which are restricted to near the ridge axis, and (2) the silicification effected by the hydrothermal circulation which acts to reduce the permeability of the rocks. Hydrothermal circulation may exist within a wide area around a spreading ridge, but it is probably confined to the higher level of the oceanic crust, where it causes continued greenschist facies metamorphism and metasomatism (Fig. 2).

Our preferred model for ocean-floor metamorphism near a spreading ridge based on the above evidence is shown in Fig. 2. Deep penetration of hydrothermal fluids is restricted to a narrow zone near the oceanic ridge, with smaller convection cells concentrated at successive higher levels away from the ridge. The greatest depth of penetration of hydrothermal circulation may depend on tectonic activity or spreading rate. Deeper penetration may occur in slow spreading ridges with well developed median valleys than in fast spreading ridges without median valleys. In Chile, metamorphic effects penetrate more deeply in ophiolites in the narrower part of the original basin which may have spread more slowly²⁴. A more complete picture of regional ocean-floor metamorphism is outlined schematically in Fig. 3. This scheme includes dynamothermal metamorphism along fracture zones which is locally complex and polyphasal, as theoretically predicted by Dewey²⁵ and later demonstrated from dredge hauls by Honnorez and Bonatti²⁶. Fracture zones also contain hydrothermal circulation systems hence hydrothermal metamorphic regimes as indicated by the presence of fresh hydrothermal metallogenic deposits²⁷. Also, burial metamorphism *sensu stricto* may play an important part in those areas where sedimentation rate and sediment thickness are high, such as along Atlantic-type continental margins²⁸ and within some marginal basins along Pacific-type continental margins.

Seismic layering and remanent magnetism

Hydrothermal metamorphism may also affect geophysical properties such as the gross seismic velocities and the magnetic properties of the oceanic crust. Models of the velocity structure and lithological character of oceanic crust have been formulated by correlating velocities measured by seismic refraction methods with compressional wave velocity measurements of rocks from the sea floor and ophiolites^{17,29}. Recent seismic work has refined the oceanic layer (layer 3) into an upper layer (3A, 6.0–6.8 km s⁻¹) and a lower layer (3B, 7.0–7.5 km s⁻¹). We suggest that the transition from metagabbros into fresh gabbros marks the transition from seismic layer 3A to layer 3B (Fig. 1). This is consistent with the compressional velocity data and conforms closely with the models of Christensen and Salisbury¹⁷ and a slightly altered model of Peterson *et al.*²⁹.

In our model, this transition marks the depth to which the hydrothermal cells along the spreading ridge have penetrated. Christensen and Salisbury¹⁷ propose that an unaltered gabbro layer is added to an original metagabbro layer by intermittent off-ridge intrusion. Another model is that layer

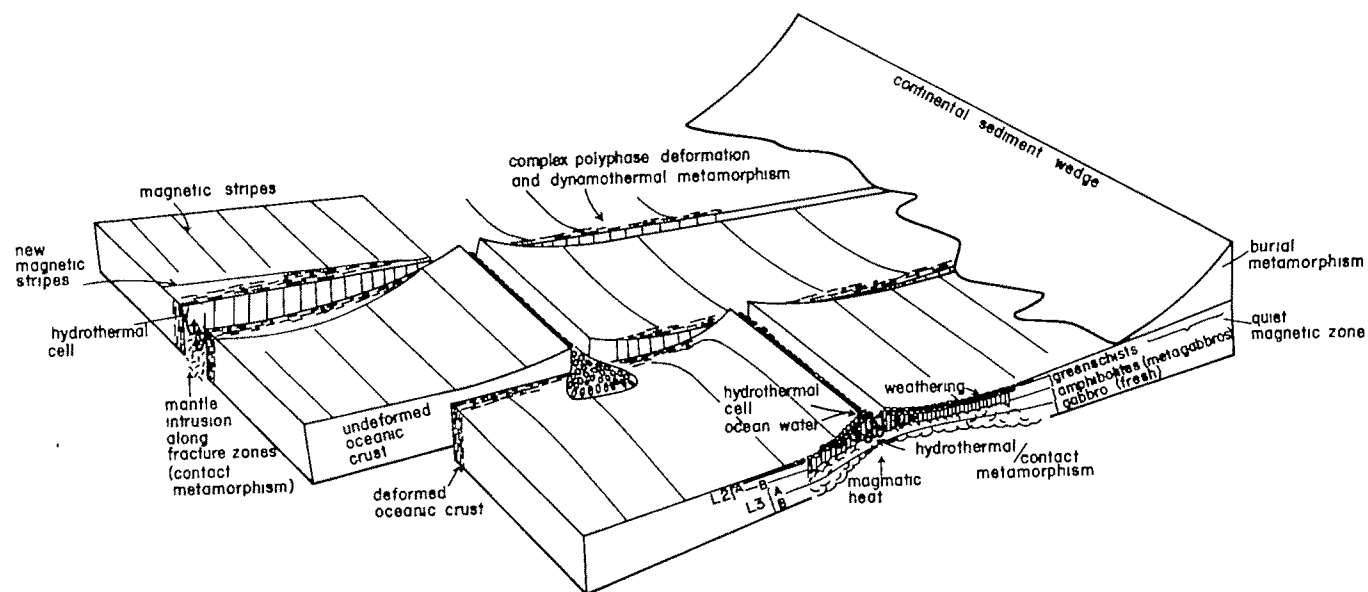


Fig. 3 Schematic illustration of the different elements of ocean-floor metamorphism, each characteristic of a distinct tectonic environment. As new ocean crust is created it undergoes hydrothermal-contact metamorphism at the ridge axis which may be overprinted at later stages by dynamothermal-hydrothermal metamorphism along fracture zones and by burial metamorphism in areas of high sedimentation

3 is initially entirely amphibolite which becomes progressively dehydrated during further spreading⁴. Our field and textural data on the ophiolite gabbros demonstrate that the opposite sequence occurs. Fresh gabbros are intruded along a central zone and their upper sections are subsequently transformed to metagabbros. This is also supported by studies on other ophiolites such as in Newfoundland (ref. 30 and J. F. Dewey and D. S. Strong, personal communication) and Troodos⁷. Off axis welding of new gabbro and cumulate ultramafic rocks probably does occur and can account for a progressive thickening of layer 3B until a steady-state thickness is reached after about 40 Myr (ref 17).

From our data in southern Chile, it is clear that both the interface between the gabbro units and the sheeted dykes, and between the sheeted dykes and the pillow lavas is complex and gradational²⁴. The original mineralogy of these overlapping zones tends however, to be homogenised as a result of the metamorphism. Therefore, we also suggest that the boundary between layers 2 and 3 (2B to 3A) marks the transition from amphibolite facies to greenschist facies rocks. Layer 2B includes the part of the sheeted dyke complex (metadolerites) and pillow lavas (metabasalts) which are in the greenschist to prehnite-pumpellyite facies of metamorphism and layer 2A is represented by the fresh to only slightly metamorphosed (zeolite facies) pillow lavas (Fig. 1). Since the metamorphic overprint on the original complex pseudo-stratigraphy of oceanic crust may be dependent on spreading rate, seismic layering may vary from ocean basin to ocean basin.

Models for the preservation of magnetic anomalies are based on measurements of the magnetic susceptibility, the intensity of natural remanent magnetisation (NRM), and the Königsberger ratio (Qn) in rocks drilled and dredged from the ocean floor and sampled from ophiolites²¹⁻²³. These measurements suggest that fresh quenched pillow basalts with high NRM and Qn are the most likely source of the magnetic anomalies (Fig. 1)²⁴. Nevertheless, even 500 m of fresh pillow lavas are not considered sufficient to produce the measured magnetic anomalies and some contribution must be made by the underlying rocks²⁵.

Our petrographic examination of ocean-floor rocks, for which Fox and Opdyke²¹ have measured the magnetic properties, shows that amphibolitised metagabbros have a higher NRM (18.3×10^{-4} e.m.u. cm^{-3} for partially amphibolitised gabbros V25-D6-39 and 69; 29.4×10^{-4} e.m.u. cm^{-3}

from strongly amphibolitised metagabbros V25-D6-17 and 56) and Qn than either greenschist facies metabasalts (0.12×10^{-4} e.m.u. cm^{-3} for all metabasalts except V25-D5-5 which is only slightly altered to a zeolite facies) and metagabbros (5.7×10^{-4} e.m.u. cm^{-3} for all metagabbros except VD-25-17 and 56 which are amphibolites) or fresh gabbros (2.1×10^{-4} e.m.u. cm^{-3} for V25-D6-6 and 27) (Fig. 1). Amphibolitised metagabbros could contribute significantly to magnetic anomalies. They contain a high concentration for skeletal growths of secondary magnetite produced by the oxidation and breakdown of olivine and pyroxene and metasomatic redistribution of Fe. Neither completely fresh gabbros nor greenschist facies rocks contain these skeletal networks of secondary opaques.

In our model of the generation and preservation of magnetic anomalies, fresh quenched pillow lavas, basalts, and gabbros all take on the magnetic polarity prevalent during their formation at the ridge axis. Hydrothermal metamorphism alters the igneous rocks, developing amphibolites with abundant secondary opaques in seismic layer 3A, and greenschist to zeolite facies assemblages in the overlying rocks. This occurs in a narrow region restricted to the spreading centre so that the polarity of the entire rock column including fresh pillows is the same. The amphibolites move rapidly out of the deeper hydrothermal metamorphic zone which is narrowly restricted to the spreading axis, effectively quenching their metamorphic textures and NRM polarity. Continued hydrothermal activity and metasomatism is confined to progressively shallower depths away from the ridge and produces greenschists with lowered NRM (Fig. 1) in the overlying rocks. This process does not affect the deeper levels within seismic layer 3A.

With time, the rock column (Fig. 1) moves away from the ridge axis. Ocean-floor weathering and shallow hydrothermal circulation continue to reduce the NRM of the pillow lavas; however, the NRM of the lower metagabbros remains constant. This effect could possibly account for the observed decrease in the amplitude of the linear magnetic anomalies away from the ridge axis for at least 10 Myr followed by a steady amplitude in the anomalies further away from the ridge (ref. 36 and W. C. Pitman III, personal communication).

Hydrothermal circulation within fracture zones could be expected to function in a fashion similar to that at a spreading ridge, although the affected rock assemblages may not be removed as quickly from the higher metamorphic zone,

except by uplift (Fig. 3). Development of secondary opaque minerals, particularly within uplifted serpentinised bodies, would cause these rocks to acquire an overprint of the prevailing magnetic polarity. Cochran²⁷ suggested that the Romanche fracture zone trough is either a zone of reduced magnetisation, or the basement is normally magnetised along the fracture zone for a distance of 500 km. We believe that magnetic overprinting could have caused the latter alternative. Young extension fracture zones, or "leaky" transforms may be expected to give high amplitude anomalies, while prolonged hydrothermal alteration during reversals will alter such anomalies significantly.

High sedimentation rates producing thick sediment blankets will induce a regime of burial metamorphism. Such thick sediment blankets are only associated with particular tectonic environments such as some types of marginal basins, as along the Western Pacific margin, and Atlantic-type continental margins (Fig. 3). The secondary metamorphism that they produce may act to retrograde any magnetic layer in the ocean crust and decrease its NRM. This effect may be one of the reasons responsible for some quiet magnetic zones along continental margins and the absence of well defined linear magnetic anomalies in some marginal basins. Larson *et al.*²⁸ presented similar arguments to account for the absence of magnetic patterns in the northern part of the Gulf of California.

A critical factor in the production of linear magnetic anomalies according to this hypothesis is the regularity of the hydrothermal metamorphism. In a marginal basin, such as the Sea of Japan, extension may not take place along a well defined linear spreading axis, but may proceed within a more diffuse zone with active regions separated by continental blocks. We expect the hydrothermal activity will be as diffuse as the igneous activity with which it is associated. We suggest that this is an additional factor that can account for the irregularity of magnetic anomalies reported within many marginal basins.

Model of metamorphic overprint

The metamorphic overprint on ophiolite complexes in southern Chile, in which metamorphic effects grade rapidly downward from zeolite to amphibolite facies and then disappear abruptly, is better explained by a model of hydrothermal metamorphism closely associated with the zone of igneous intrusion than by the classical concept of regional burial metamorphism. The metamorphic conditions that are reached during this metamorphism are principally a function of temperature and fluid phases. Hence, if the present metamorphic facies concept is to be continued and extended to include ocean floor metamorphism, metamorphic facies must be plotted on three-dimensional diagrams in order to include fluid phase variables, principally H₂O.

The metamorphic overprint may have a more significant influence than the igneous pseudo-stratigraphy in determining some marine geophysical properties. Irregularities in the juxtaposition of the overprint on the pseudo-stratigraphy caused by various rates of spreading, sedimentation affecting hydrothermal circulation, and the linearity or irregularity of spreading activity determined by tectonic environment, all influence the variability of the geophysical properties of the rocks.

The overprint of the metamorphism determines the seismic layering of the ocean floor, seismic layer 2A consists of fresh, weathered, and zeolite facies pillow lavas, basalt and dolerite dykes; seismic layer 3A consists of amphibolite facies dolerite dykes and metagabbros, seismic layer 3B consists of fresh gabbros intruded at the ridge axis but below the greatest depth of penetration of the hydrothermal metamorphism.

The deepest levels of the metamorphism produce an oxidation zone with extensive development of secondary

fine grained opaque minerals which may enable rocks in this zone to preserve a stronger NRM than both underlying fresh gabbros and overlying greenschist, zeolite, and weathered basalts. Marine magnetic anomalies are likely to be produced by an integrated effect of layer 2A and 3A. Layer 3A is not affected by off-axis shallow-level hydrothermal circulation which weathers and spilitises the overlying basalts reducing their NRM. Thus with time this layer may become a more significant contributor to magnetic anomalies.

Irregularities in magnetic anomalies may be related to irregularities in ocean-floor metamorphism caused by (1) high sedimentation such as found along Atlantic-type continental margins, and in some marginal basins, which promotes a new overprint of classical burial metamorphism creating one type of magnetic quiet zones and, (2) diffuseness of the zone of extension such as in incipient rift zones or marginal basins which will result in an irregular pattern of hydrothermal circulation, thus decreasing the likelihood of the generation and/or preservation of linear magnetic anomalies.

Mantle intrusion into extended fracture zones sets off independent regimes of hydrothermal/contact metamorphism and consequently overprints earlier magnetic anomalies in the vicinity of the fracture zones. Magnetic anomalies associated with such fracture zones are expected to be complex, ranging from very high after initial emplacement of uplifted serpentinites to low following extended metamorphism during magnetic reversals.

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Reassessment of roles of oxygen and ultraviolet light in Precambrian evolution

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The rise of atmospheric oxygen occurred long before the sudden appearance of multicellular eukaryotic organisms in the later Precambrian. Oxygen was necessary but not sufficient for the evolution of multicellular eukaryotes: the rise of modern aerobic eukaryotes (fungi, animals and plants) occurred in a fully oxygenic atmosphere only after the evolution in protists of microtubule-utilising processes (mitosis and meiosis).

ALTHOUGH the Earth probably formed about 4.5×10^9 yr ago the oldest rocks found to date are only about 3.5×10^9 yr old¹. Nearly as old as the oldest rocks themselves is the earliest evidence for microbial life, both lithified fossil communities of microorganisms (stromatolites) and silicified remains of bacteria and blue-green algae can be found in sedimentary rocks at least 3×10^9 yr old^{2,3}. Nevertheless the fossil record is extremely sparse during the early period of Earth history. It is not until the beginning of the Phanerozoic era, about 5.8×10^7 yr ago, that a diversity of metazoan and other species suddenly becomes abundant in the fossil record. This abundance of fossils has continued throughout subsequent geological history.

Various explanations have been offered for the sparsity of the Precambrian fossil record and the sudden appearance of abundant fossils at the beginning of the Phanerozoic. The simplicity of two of these suggestions has made them particularly popular. Both are related to the transition from a primitive reducing atmosphere to the modern atmosphere, rich in oxygen. One suggestion⁴⁻⁶, is that atmospheric oxygen became sufficiently abundant to support respiring organisms, including skeleton-forming metazoa, only near the end of the Precambrian. The other suggestion^{4,8} is that Precambrian life was confined to very restricted habitats by the flux of solar ultraviolet radiation that would have bathed the surface of the Earth when the atmosphere lacked sufficient oxygen to produce an ultraviolet screen of ozone. The development of an ozone screen near the end of the Precambrian is presumed to have made possible, for the first time, widespread colonisation of the open ocean accompanied by diversification of life and subsequent formation of abundant fossils.

We argue here that neither of these explanations for the sudden appearance of fossils at the beginning of the Phanerozoic can be accepted. Both geological and biological evidence suggest that the transition from a reducing atmosphere to an oxidising atmosphere occurred long before the end of the Precambrian. In addition, while the metabolic capabilities of prokaryotic organisms (bacteria and blue-

green algae) suggest that they evolved under conditions of changing oxygen tension, the response to oxygen of eukaryotic organisms (which include fungi, animals, complex algae, and plants) is uniform; nearly all of them are aerobic. The implication is that the transition to an oxidising atmosphere preceded the origin of the eukaryotic cells, which in turn must have preceded the origin of metazoa. The accumulation of oxygen in the atmosphere well before the end of the Precambrian would have eliminated solar ultraviolet light as a significant biological factor at the end of the Precambrian. Moreover, the number of methods by which organisms can protect themselves from harmful ultraviolet radiation is sufficiently large to suggest that solar ultraviolet, even when the atmosphere was anaerobic, was more of a tolerable annoyance than a lethal agent that controlled the distribution and diversification of life.

We offer an alternative explanation for the late and apparently sudden appearance of metazoa in lower Cambrian sediments. Our explanation is related to the mechanisms by which fully mature eukaryotic cells (precursors to the metazoa) most probably originated⁹⁻¹¹. There was probably a protracted evolution of modern genetic systems based on mitosis in cells which acquired certain organelles (for example, plastids and mitochondria) by hereditary endosymbiosis^{9,10}. We concur with Cloud¹² that it is the origin of hard parts that underlies the Cambrian explosion of metazoans and we invoke biotic factors intimately associated with the early evolution of eukaryotes to explain the relatively late appearance of metazoa, metaphyta, and fungi.

The rise of oxygen in the atmosphere

It is not likely that abiotic sources of oxygen could have produced an aerobic atmosphere before the origin of oxygen-producing photosynthesis. The only significant abiotic source of atmospheric oxygen¹³ is provided by photolysis of water vapour in the upper atmosphere followed by escape of hydrogen to space. Berkner and Marshall¹⁷ and Brinkmann¹⁴ made estimates of the level of oxygen in the prebiological atmosphere that could have been maintained by this source. These estimates are almost certainly too high. Both studies erred by assuming that every photolysis is followed by escape. Today, only a small fraction (about 10%) of the hydrogen atoms produced on Earth by photolysis escape. Most of them recombine to form water, leaving no free oxygen. This is true not only in the oxidising atmosphere of the Earth, but also in the less aerobic atmospheres of Mars and Venus¹⁵.

The processes that control the rate of escape of hydrogen from planetary atmospheres have been described by

Hunten¹³ Under present conditions, as well as under conditions that are likely to have existed in the primitive terrestrial atmosphere, escape is controlled not by the rate of photochemical production of hydrogen but by the rate at which hydrogen and its compounds are transported upwards from the lower atmosphere to the exosphere. This rate is proportional to the abundance of hydrogen compounds (principally water vapour) in the stratosphere. The abundance of water in the stratosphere is controlled by condensation at the tropopause¹⁴ The abiotic source of oxygen therefore depends on the temperature of the tropopause and not on the rate of photolysis of water vapour. Unless the primitive tropopause was warmer than the present one the abiotic source of oxygen in the primitive atmosphere would have been no larger than it is today. The sources estimated by Berkner and Marshall¹ and by Brinkmann¹⁴ were considerably larger.

In estimating the oxygen budget of the primitive atmosphere these authors also erred by neglecting oxygen consumption in reactions with reduced volcanic gases, principally hydrogen. Today the volcanic hydrogen source seems to be comparable to the rate of escape of hydrogen from atmosphere to space. It is likely to have been larger in the early Precambrian because accretional and radioactive heating should have caused higher temperatures within the Earth. If the volcanic source of hydrogen was larger than the abiotic source of oxygen, then atmospheric oxygen would have been held at very low partial pressures by photochemical reaction with an excess of atmospheric hydrogen. This was presumably the situation when life originated.

The origin of cyanobacterial photosynthesis between 2 and 3×10^9 yr ago introduced a new source of atmospheric oxygen. (Cyanobacteria are blue-green algae¹⁷, from the point of view of photosynthetic oxygen elimination cyanobacterial photosynthesis is the same as green-plant photosynthesis.) This source presumably increased with time. As the interior of the Earth cooled down, moreover, the volcanic hydrogen source may well have decreased. Eventually there came a time when the rate of release of oxygen to the atmosphere exceeded the rate of release of hydrogen. When this threshold was reached the earlier excess of hydrogen would have been replaced by an excess of oxygen. The hydrogen partial pressure would have been driven to low values by photochemical reaction with oxygen. The transition from a reducing atmosphere to an oxidising one could have been quite rapid on the geological time scale.

The time of this transition has been dated at $2.0 \pm 0.2 \times 10^9$ yr ago by Cloud¹⁸. This is the time when red beds replaced banded iron formations in the sedimentary record. Deposition of widespread banded iron formations probably required an anaerobic atmosphere¹⁸; deposition of red beds required an aerobic one¹⁹. How much oxygen is required for the deposition of red beds does not seem to be known. There is, however, biological evidence that the atmosphere contained enough oxygen to support aerobic metabolism well before the origin of metazoa. In fact, Dimroth and Kimberley²⁰ have seriously challenged Cloud's conclusions and have argued that it is even possible that fully oxygenic atmospheric conditions prevailed as far back as the Archean (more than $2,500 \times 10^6$ yr ago).

Genetic variation in related taxa of organisms (for example, species, genera, and families) that can be understood in terms of significant environmental variables can often give insight into the nature of the selection pressures incumbent on the population at the time speciation occurred. Thus, the origin of asymmetry in the flowering parts of angiosperms can be understood in terms of their co-evolution with pollinating mammals, birds and insects; the development of hydrodynamic form in several mammalian lines (dolphins, seals, sea lions, manatees, and whales) can be comprehended as an evolutionary response to increasing periods of the life cycle in aqueous habitats.

The analogous variable response of prokaryotic microorganisms to ambient oxygen is consistent with the view that, as a group, they evolved their major metabolic patterns in response to varying and rising oxygen tensions. Prokaryotes include obligate anaerobes, facultative anaerobes, microaerophils, oxygen-indifferent organisms, and obligate aerobes. Closely related microbes often differ markedly in their response to oxygen, suggesting that evolution of oxygen-handling mechanisms evolved convergently in several prokaryote groups.

By contrast, eukaryotes are extremely uniform in their response to oxygen. They are aerobes. Obligate anaerobes are extremely rare among eukaryotes; there are no metazoan animals or embryophyte green plants that complete their entire life cycles in the total absence of oxygen. No oxygen-indifferent forms have been reported. Among the fungi, a very few are anaerobic and all of these are facultatively aerobic. All eukaryotic anaerobes seem to be derived secondarily from aerobic ancestors. The yeasts, for example, survive and grow fermentatively by reversibly dedifferentiating their mitochondria. Even in anaerobic growth conditions they require metabolites made by aerobic, oxygen-utilising pathways (for example, polyunsaturated fatty acids and sterols). Among the protists only a very few obligate anaerobes or microaerophils are known; some psammophilic ciliates and a number of polymastigote flagellates which, judging from their aerobic, mitochondria-containing, close relatives, seem to have lost their oxygen-utilising mitochondria. Only those that have replaced the lost mitochondria with symbiotic bacteria are able, it seems, to produce the nearly ubiquitous intracellular membranous differentiation known as Golgi apparatus²¹. Furthermore, all of these flagellates are symbiotic forms, primarily in the anaerobic hind-guts of termites. It is surmised that oxygen-requiring metabolites are provided to the symbionts by their hosts or in the diets of the hosts.

The process of mitosis itself requires molecular oxygen²² as does the formation of steroids, polyunsaturated fatty acids that comprise eukaryotic membrane systems, the amino acids tyrosine (at least in animals) and hydroxyproline, as well as hydroxyproline-rich proteins such as collagen and other typical eukaryotic metabolites. Mitochondrial metabolism also involves many oxygen-requiring steps (for example, the squalene oxidase-catalysed production of lanosterol, tyrosine formation, and so forth). It therefore seems likely that full aerobiosis was characteristic of even primitive eukaryotes.

While oxygen was necessary for eukaryotic origin and evolution, there is no evidence that the mere appearance of atmospheric oxygen was sufficient. Complete and obligate aerobiosis is fully documented in many species of prokaryotes²³. As oxygen is depleted both in laboratory and natural communities of microorganisms (on, say, a depth gradient in soil) a distinct correlation can be made of the distribution of the microbial species along that oxygen gradient. Oxygen gradient distribution is less prevalent among the more abundant eukaryotes: they tend to be associated only with fully aerobic or even oxygen supersaturated environments. An easy method to select for prokaryotes and against eukaryotes is simply to fill a container up to overflow with nutrient solutions and place a lid on it. The most reasonable explanation for these phenomena is that oxygen was and still is a selective agent among prokaryotes but that close to modern quantities of oxygen are now and always have been required for eukaryotes. This is consistent with the idea of evolution of major fermentative and respiratory pathways in prokaryotes before and during the transition to the oxidising atmosphere²⁴ and the origin of eukaryotes at a later time.

The origin of the eukaryotic cell after the transition to an oxidising atmosphere, but several hundred million years

at least before the end of the Precambrian^{25,26}, supports the geological evidence that the transition occurred well before the end of the Precambrian. The sudden appearance of abundant fossils at the end of the Precambrian was therefore not associated with the onset of an aerobic atmosphere. The biological evidence, moreover, directly contradicts the idea that the sudden appearance was a result of rising levels of atmospheric oxygen. Prokaryote metabolism appears to have evolved during a period of rising oxygen; the origin of eukaryotic cells, which must have preceded the origin of metazoa composed of these cells, appears to have occurred in an aerobic environment.

Evolutionary effects of ultraviolet light

Biologically harmful solar ultraviolet radiation is absorbed in the terrestrial atmosphere by ozone, produced photochemically from atmospheric oxygen. This ozone screen must have developed gradually as oxygen accumulated in the atmosphere, leading to a changing radiation level in the environment of primitive organisms.

The variation of ozone abundance with the oxygen partial pressure is a problem that would merit study with one of the elaborate photochemical models that have been developed for the investigation of man's impact on the stratosphere²⁷⁻²⁹. Such a study has not yet been conducted, however.

Berker and Marshall⁴ in their investigation of surface ultraviolet during the rise of atmospheric oxygen, made no attempt to solve the problem. They simply assumed a decrease of ozone with oxygen. Ratner and Walker³ used a very simple photochemical model (oxygen reactions only and no transport). They found that the first effect of a decrease in oxygen below present levels is an increase in ozone. The increase occurs because ozone is formed by a three-body reaction which becomes more rapid as the ozone layer moves to lower altitudes. The result is that substantial densities of ozone can exist at quite low oxygen partial pressures. Ratner and Walker found that the atmosphere would contain enough ozone to shield the ground from solar ultraviolet radiation at oxygen partial pressures only 10^{-3} times their modern values. This conclusion might be modified by a study that considered the effects of nitrogen oxides, chlorine, and methane on the ozone photochemistry, but it is impossible to guess whether these complications would increase or decrease the ozone abundance. At present, the best we can do is accept the result of Ratner and Walker³ and conclude that the ozone screen was established before the abundance of atmospheric oxygen reached 1% of its present level. It is clear from the fossil record^{4,31} that this level was reached before 7×10^8 yr ago. Ultraviolet light therefore may well have disappeared from the surface of the Earth long before the end of the Precambrian. In addition, the biological evidence that eukaryotic cells evolved in an aerobic world indicates that ultraviolet light had disappeared from the surface of the Earth even before eukaryotes evolved. We may infer that biologically harmful solar ultraviolet radiation was probably not a factor either in the early evolution of eukaryotic organisms or in the sudden appearance of abundant metazoa at the beginning of the Phanerozoic era.

It is possible, however, that ultraviolet affected the evolution of prokaryotic organisms at even earlier times, before the transition to an oxidising atmosphere. Sagan³² has examined the flux of solar ultraviolet radiation at the ground in a primitive atmosphere devoid of ozone. An unprotected microorganism would be killed in a matter of seconds if exposed to full sunlight. Some scattering and attenuation of radiation by Rayleigh scattering, dust, and water droplets is to be expected³³ but not enough to render the surface habitable. Sagan has shown that an ultraviolet screen could not have been provided by methane, ammonia,

water vapour, carbon dioxide, nitrogen, hydrogen, or hydrogen sulphide. None of these gases absorbs sufficiently strongly at wavelengths near 2,400 Å. Gaseous organic molecules have also been considered by Sagan. They are not likely to have been sufficiently abundant. Sulphur dioxide does absorb strongly throughout the near ultraviolet^{34,35} but its presence is doubtful in the Archean anoxic atmosphere.

The possible screening of organisms by liquid water has been considered by Berkner and Marshall and by Sagan. Pure water absorbs weakly in the ultraviolet; a water thickness of at least a metre would have been needed to protect unshielded organisms in an ozone-free atmosphere, rich in hydrogen sulphide. More than 10 m of water would have been necessary if the atmosphere lacked hydrogen sulphide. On the basis of this work we conclude that inorganic ultraviolet screens were ineffective in the anoxic atmosphere. It is possible that prokaryotic microorganisms, including those that required visible light, may have lived at a safe depth below the surface of the sea, but it is more likely that they used a number of biological mechanisms to protect themselves from ultraviolet damage.

Prokaryotic microorganisms are able to protect themselves against potentially lethal ultraviolet radiation by a variety of means, ranging from avoidance of radiation to enzyme-mediated repair of radiation damage. Negative phototaxis (swimming away from the light source) is one of these mechanisms. It is a behaviour that is known in heterotrophs and non-obligate photoautotrophs as well as microbes as primitive as clostridia, desulphovibrios, and purple non-sulphur photosynthetic bacteria.

Blue-green algae and certain bacteria tend to grow in mats. As suggested by Monty³⁶ it is likely that the matting habit evolved, at least in photoautotrophs, as a protective mechanism against solar ultraviolet light. For example, we have recently observed that large inoculum size (which is equivalent to the matting habit) can protect from death underlying cells in clumps of ultraviolet irradiated blue-green algae. These studies involved *Lyngbya*, very commonly a surface component of algal mat communities. Even after 3 d of continuous ultraviolet irradiation (2×10^5 erg mm⁻², $\lambda_{\text{max}} = 254$ nm), cells on the interior of a mat were entirely viable in conditions in which controls on the surface were killed after minutes^{37,38}. The most common Precambrian fossils, lithified remains of algal mat communities (stromatolites), suggest that the matting habit has been in continuous existence since more than 3×10^8 yr ago³⁹. The surface components of these layers of microbial communities must have afforded excellent protection to underlying layers against potentially lethal solar ultraviolet radiation.

A third method of protection against ultraviolet radiation was discovered in the same experiments. Solutions of simple nitrogenous salts (sodium nitrate and sodium nitrite) absorb strongly in the near ultraviolet. The addition of these salts to the medium in which the algae were immersed was found to protect small amounts of surface filamentous algae from death by irradiation^{37,38}. Survival has been extended greatly by the matting habit or the presence of these nitrogenous salts even in *Lyngbya* directly exposed to the full unfiltered ultraviolet spectrum from a 300-W deuterium lamp at a distance of only 13 ± 0.5 cm (C. Walters, unpublished observations). These protective effects were measured in conditions that prevented photoreactivation. Yet it is well known that ultraviolet damage to nucleic acids calls into play enzymatic repair systems mediated by visible light (photoreactivation). Light-insensitive (dark) repair systems are also known. Such ultraviolet repair systems are apparently universally distributed among bacteria, providing still another mechanism that would have permitted primitive prokaryotic organisms to flourish even in the absence of an atmospheric screen against solar ultraviolet radiation.

The continued presence in many organisms of enzymatic systems for the repair of ultraviolet damage in the absence of any danger of ultraviolet damage has been attributed to the association between ultraviolet sensitivity and lesions in recombination; there is at least one component in the enzyme systems of *E. coli* that controls DNA recombination (breakage and reunion of parent DNA molecules in the formation of new daughter recombinant molecules) and that is also involved in repair after ultraviolet irradiation³⁹. Thus, natural selection for the retention of sexual recombination and DNA repair systems has led to a co-selection for a Precambrian legacy of protection against solar ultraviolet radiation. Bacterial and blue-green algal mutants with high resistance to ultraviolet light have also been reported⁴⁰.

Environmental, behavioural, physiological, and genetic mechanisms for resistance to potentially dangerous ultraviolet light have therefore been established. It is possible that only a fraction of the protection mechanisms have been discovered, but it is clear that a wide range of responses exist even in extant populations in which the threat of dangerous natural ultraviolet irradiation no longer exists. We conclude that potentially lethal solar ultraviolet radiation was a source of selection pressure that led to the stabilisation and retention of several protection mechanisms during and immediately after the origins of prokaryotic cells that it had little effect on the later origin and evolution of eukaryotic cells and higher organisms.

Late appearance of multicellular eukaryotes

Mitosis may be considered to be a process that is a precursor to the sexual and developmental systems of multicellular organisms. Such sexual and developmental processes are based on meiotic-fertilisation life cycles, and meiosis is, in essence, a highly specific and directed variation of mitosis. It is probable, therefore, that the late appearance of metazoan, metaphytan and fungal organisms is, at least in part, a result of the complexity of the mitotic-meiotic system, and the length of time required for it to evolve.

Recent studies on the variations in mitosis and meiosis suggest that these processes evolved in eukaryotic microorganisms, primarily heterotrophs⁴¹. The variations in the mitotic systems of amoebae, dinoflagellates, flagellate algae, radiolarians, ciliates, and amoeboflagellates suggest that stabilisation of the mitotic-meiotic system was a prerequisite for the impressive morphological diversification of metazoa and metaphyta. This stabilisation most probably involved many complicated steps as determined by significant variations in mitosis and sexual life cycles within groups of closely related eukaryotic microorganisms: dinoflagellates⁴²; chlorophytic algae⁴³; hypermastigote and other flagellates⁴⁴⁻⁴⁶; amoebae and amoeboflagellates⁴⁷; ciliates^{48,49}; flagellated fungi⁵⁰; and cellular slime moulds⁵¹.

These steps include, for example, the origin of the nucleolar cycle; chromosomal organisation involving histone, non-histone proteins and chromatin RNA; the development of the chromosomal coiling cycle; the development of microtubule-mediated gene distribution mechanisms (including the origins of kinetochores, centrioles or spindle plaques, and spindle pole bodies); the origin of sex-determining mechanisms, sexual attraction systems, syngamy, nuclear fusion, synapsis, bivalent formation and reduction division. It is plausible to assume that the origin and stabilisation of these elaborate cell division mechanisms evolved in the group of organisms that still shows variations: the eukaryotic microorganisms. There has always been a general consensus among botanists and zoologists that plants and animals are descendants of these protists. We may assume then that mitosis followed by meiosis arose in protists sometime between 2 and 0.7×10^9 yr ago, after the transition to the oxygenic atmosphere. Because of their complexity these processes may have taken a long time to evolve; their

development must have preceded the origin of most multicellular eukaryotes. We therefore attribute the late appearance of metazoa, metaphyta and fungi to primarily biological rather than environmental factors.

It has recently been established that mitotic microtubule systems require low and regulated calcium ion concentrations for polymerisation of the component tubulin protein into tubules⁵². Thus, it is possible that the removal of calcium and its external deposition evolved primarily for the intracellular stabilisation of the mitotic apparatus and other somatic microtubules, thereby preadapting many organisms for the formation of protective and supportive calcium carbonate hard parts. This idea is supported by the observation that microtubules of the axopods of multi-chambered foraminiferans are involved in the calcium carbonate deposition of the shells⁵³. According to this idea, calcium carbonate depositional systems generally require intracellular interactions with microtubules. Calcium carbonate shell deposition could easily have originated in populations of protists and metazoan organisms that regulated intracellular calcium ion concentrations in carbonate-rich waters.

Environmental and biological factors

In summary we suggest the following sequence of coupled environmental and evolutionary changes during the Archean and Proterozoic eras: the origin and diversification of anaerobic bacterial life that developed many mechanisms of ultraviolet avoidance, tolerance, and protection; the origin of oxygen-eliminating photosynthesis in cyanobacteria (resembling their modern counterpart, *Oscillatoria limnetica*⁵⁴), microorganisms that utilised atmospheric ammonia, nitrogen, or reduced organic nitrogen as sources of nitrogen for protein synthesis. The evolution of physiologically modern blue-green algae led to the release of oxygen into the environment, primarily by blue-green algal mat communities. The existence of these communities is inferred from their lithified remains—Precambrian stromatolites. Photosynthetic oxygen production permitted the abiological formation of nitrites and nitrates as well as the accumulation of oxygen in the atmosphere. The use of nitrate and nitrite as still another mechanism of protection against ultraviolet irradiation may have led to the origin of nitrate reductive pathways as a source of cellular nitrogen which possibly were precursors of aerobic respiration^{55,56}. The evolution of major metabolic pathways included the origin of facultative and obligate aerobiosis in prokaryotes.

The diversification of prokaryotes was accompanied by the evolution of symbiotic interactions between groups of microorganisms including those that were ancestral to modern eukaryotes. The rise of modern aerobic eukaryotes, including many lines of multicellular forms, then followed only after the evolution of microtubule-utilising mitosis and meiosis. The origin of mitotic and eventually meiotic systems occurred in various lines of eukaryotic protists. The speciation events that produced major groups of multicellular eukaryotes (plants, animals, and fungi) occurred in an oxygenic world⁵⁵.

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Evaluation of six short term tests for detecting organic chemical carcinogens and recommendations for their use

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Six short term tests for detecting carcinogenicity have been evaluated using 120 compounds, of which half were carcinogens and the rest non-carcinogens. The results obtained indicate that the Ames test and a "cell transformation" assay are both sufficiently sensitive to carcinogenicity, or the lack of it, in the compounds studied to enable them to be employed for detecting potential carcinogens. The consequences of using short term tests under various screening conditions have been explored. In order to have confidence in the results obtained for new or previously untested compounds it is important to use such tests in a carefully controlled manner.

Two methods are commonly used for predicting the potential human carcinogenicity of an organic chemical. The first is to examine its chemical structure for similarities to those of known human or experimental carcinogens, and the second, which usually follows from a positive correlation in the first, is to undertake animal carcinogenicity studies. Although this approach has revealed many new carcinogens it is time consuming and expensive, and further, it tends to identify new analogues of established carcinogens rather than carcinogens of novel structure. It is, therefore, not surprising that clinical observations or epidemiological evidence have sometimes given the first indication that a particular chemical is a human carcinogen. Such cases, as exemplified by the increased incidence of bladder cancer among both benzidine¹ and β -naphthylamine² workers, have usually been limited to small groups of people in the particular industry or activity concerned, and although each one has been a serious individual hazard they collectively contribute only a small part to the total cancer burden of the population at large.

It has been suggested by several scientists in this field that a significant proportion of the 'spontaneous' human cancer in-

cidence is dependent on environmental factors, one of which may be the presence of both natural and contaminant carcinogens in the environment^{3,4}, however, to test every environmental and industrial chemical for carcinogenicity by conventional methods would clearly be impossible within the foreseeable future. It is mainly for this reason that attempts are being made to develop short term tests for potential carcinogenicity.

A variety of short term tests have been described (see ref. 5 and refs therein), but only one, the Ames test (ref. 6 and refs therein), has been evaluated in any detail. We therefore undertook an evaluation of a variety of established and novel short term tests to determine their strengths and limitations and to decide if any one test, or a combination of them, could form the basis of a screen. In addition, we needed to consider how such a screen could most effectively be used to avoid the development of unrecognised potential human carcinogens in the future and also to detect any unsuspected carcinogens which might still be in use.

Evaluation of the chosen short term tests

Ten short term tests have been evaluated. The choice of tests was decided partly on empirical grounds and partly by the need to work within the resources made available for this study. Thus, the exclusion of other available tests from this study does not mean that they should automatically be excluded from any future carcinogen screens. A brief evaluation of the iodine⁸ and the acridine⁹ colour tests, the transplacental blastomagenesis test¹⁰ and the piperidine alkylation test¹¹ was sufficient to establish that they were unsuitable for our purposes, and consequently, they were not pursued further.

The main evaluation was of the six tests described below and was carried out using 120 organic chemicals, 58 of which are known human or animal carcinogens, the remaining being putative non-carcinogens. The difficulties implicit in such classification have been commented on elsewhere⁵ and the guidelines developed for this study will be described in detail later. The compounds about

Table 1 Results of six short-term tests for carcinogenicity

	Polycyclics	Arylamines	Alkylating agents	Miscellaneous	Total
1 Ames test	95	94	83	94	93
2 Cell transformation	95	97	94	92	94
3 Rabin's test	65	76	61	73	71
4 Subcutaneous implants	88	60	25	81	68
5 Sebaceous gland suppression	90	62	67	57	65
6 Tetrazolium reduction	50	56	39	67	57

120 compounds (carcinogens and non-carcinogens) were tested. The figures refer to the percentage of accurate predictions made by each test for individual classes of compounds and for the total group.

which any doubt existed, such as trimethyl phosphate were, however, few and a change of their classification would not substantially affect the overall results

The compounds were chosen to represent a wide range of carcinogens and non-carcinogens and have been somewhat arbitrarily subdivided into chemical classes. Where possible, structurally related carcinogen and non-carcinogen pairs were included in the study. The compounds were coded and each was tested as a solution in either dimethyl sulphoxide (DMSO) or water without operator knowledge of their individual biological activity. Each compound was tested once in each of the test systems and no attempt was made to optimise the test conditions for a particular compound, nor to determine the reproducibility of individual results. A detailed presentation of this study will be made at a later date, but the following is a summary of the methods employed and the results obtained

● Ames test. Compounds were tested using four strains of *Salmonella typhimurium* (TA 1535, 1538, 98 and 100) in the plate incorporation assay of Ames⁶. A rat liver microsomal preparation (S-9 fraction: cofactor, 1:3) was used⁶.

● Cell transformation. A novel technique has been devised in which neonatal Syrian hamster kidney fibroblasts (BHK21/C13) and either human diploid lung fibroblasts (WI-38) or human liver cells (Chang) were exposed to five different doses of the test compounds *in vitro* in liquid tissue culture medium without serum. The S-9 mix of the Ames test⁶ was included in the culture medium to aid in the metabolism of the test compound. To assess survival following exposure to a compound a small number of cells from each treated sample were grown in liquid medium and colonies counted after 6–8 d incubation. A dose-response curve for survival was constructed and the LD₅₀ calculated. The remaining cells were transferred to semi-solid agar which permitted the growth of transformed cells¹². A dose-response curve for transformation was constructed and the number of transformed colonies at the LD₅₀ dose calculated. A 2.5 times increase in colonies over controls was regarded as positive. Although this test is referred to as cell transformation, growth in semi-solid agar is only one of the accepted criteria for cell transformation

● Rabin's test. The loss of ribosomes from isolated rat liver rough endoplasmic reticulum (RER) following incubation with potent carcinogens *in vitro*, as first described by Williams and Rabin¹³, has been quantitatively monitored by radio-tracer techniques¹⁴. An increase (10% or greater) in degranulation of the RER by the test compound, as compared with negative controls, was taken to indicate a positive result

● Implant test. A novel technique has been developed based on the histological assessment of the fibrous capsule and surrounding tissues which develop three months after surgical implantation in mice of Millipore filter disks overlain with a gelatinous suspension of the test compound

The histological appearance of the capsule surrounding each implant was scored on a scale between 1 (low) and 5 (high) based on the appearance of lesions thought to be indicative of neoplasia. The mean scores from control implants containing only DMSO and gelatin were then compared with those from the test implants. Compounds causing a statistically significant increase in the mean score were taken as positive in the test

● Sebaceous gland test. Bock and Mund have demonstrated that the sebaceous glands of mouse skin are sensitive to the topical

application of carcinogens¹⁵. Test chemicals were applied directly to mouse skin and those causing a statistically significant decrease in the ratio of sebaceous glands to hair follicles were taken to be positive.

● Tetrazolium reduction test. The test was based on that described by Iversen and Evensen¹⁶. Samples of mouse skin which had been exposed to the test compound *in vivo* were incubated in aqueous solutions of tetrazolium red. Statistically significant increases in the *in situ* biological reduction of the colourless tetrazolium compound to the coloured formazan were measured spectrophotometrically and taken to indicate a positive response for the test compound.

A summary of the results obtained in this study is given in Tables 1–3. An overall impression of the performance of each of the tests can be gained from the 'total' column of Table 1, which shows the percentage of correct predictions made by each test with the complete group of 120 compounds. It is evident that only the first two tests performed well. The remaining columns of Table 1 show how each test performed with the individual chemical classes of compounds. With the exceptions of the response of test 4 to alkylating agents and test 5 to polycyclic aromatic hydrocarbons, the predictive accuracy of each of the tests is essentially independent of the chemical class of the test compound. However, it is probable that a different study using different test compounds from those used here would give slightly different overall performance figures.

An alternative method of assessing the results produced by the various tests is shown in Table 2. For a test to be useful for either research or screening purposes it must be capable of detecting a

Table 2 Results from the six short term tests expressed as the percentage of accurate predictions for 58 carcinogens and 62 non-carcinogens

CARCINOGENS		
1 Ames test		91
2 Cell transformation		91
3 Rabin's test		71
4 Subcutaneous implants		37
5 Sebaceous gland suppression		67
6 Tetrazolium reduction		40
NON-CARCINOGENS		
1 Ames test		93
2 Cell transformation		97
3 Rabin's test		71
4 Subcutaneous implants		95
5 Sebaceous gland suppression		64
6 Tetrazolium reduction		73

Table 3 Response of the six short term tests to eight carcinogen and non-carcinogen pairs

Test compound	Ames test	Cell transformation	Rabin's test	Subcutaneous implants	Sebaceous gland suppression	Tetrazolium reduction	Animal carcinogenicity
4-Nitroquinoline- <i>N</i> -oxide	+	+	+	+	+	+	+
3-Methyl-4-nitroquinoline- <i>N</i> -oxide	—	—	—	—	—	—	—
Benzidine	+	+	+	—	+	—	+
3,3',5,5'-Tetramethylbenzidine	—	—	+	—	—	—	—
2-Acetylaminofluorene	+	+	+	—	—	—	+
4-Acetylaminofluorene	—	—	+	*	*	*	—
9,10-Dimethylantracene	+	+	—	+	+	+	+
Anthracene	—	—	+	—	—	—	—
Dimethylcarbamoyl chloride	+	+	—	+	+	—	+
Dimethylformamide	—	—	—	—	—	—	—
1-Fluoro-2,4-dinitrobenzene	+	+	—	+	+	—	+
1,3-Dinitrobenzene	—	—	—	—	+	—	—
β -Naphthylamine	+	—	+	—	+	+	+
α -Naphthylamine	—	—	—	—	—	—	—
Nitrosolic acid	+	+	+	—	—	—	+
Diphenylnitrosamine	—	—	+	—	+	+	—
Number of pairs correctly identified	8	7	2	4	5	3	

* Not tested

high proportion of any carcinogens which may be present in a group of compounds, and in doing so generate as small a number of false positives as possible (a false positive is defined here as a positive result obtained in a test system with a compound which has been tested for carcinogenicity in animals and found to be negative) Table 2, therefore, records the percentage of correct predictions made by each of the tests in the two complete subgroups of carcinogens and non-carcinogens and it is clear that only the first two tests possess the required balance of response. Obviously, the lower the detection rate of a test to non-carcinogens, the higher the number of false positives produced. The remaining four tests were generally less reliable. For example, tests 3 and 5 correctly identified 71% and 67% respectively of the test carcinogens but in doing so they generated a high proportion of false positives (29% and 36% respectively). This would therefore reduce the reliance that could be placed on a positive result generated by either of these tests for a compound of unknown biological activity. In contrast, test 4 correctly identified only 37% of the test carcinogens and generated a low proportion of false positives (5%). While these results make tests 3–5 of little value in isolation for screening purposes they may be of value when used in combination with other tests or for assessing the likely carcinogenic potency of a potential carcinogen. Test 6 performed poorly in all respects and is, therefore, of no general value.

The results shown in Tables 1 and 2 clearly establish that the Ames test (test 1) and the cell transformation assay (test 2) are both able to detect a high percentage of a wide range of carcinogens while also generating an acceptably low level of false positives. The usefulness of these two tests is further underlined by the selected results shown in Table 3. These illustrate that with an exception, that of test 2 with the naphthylamines, both are capable of correctly distinguishing between the eight structurally related carcinogen and non-carcinogen pairs used in this study. It is of importance to any screening programme that might use either of these tests that they have been shown to be sufficiently sensitive to register, for example, the weak carcinogen 9,10-dimethylantracene as positive while finding the parent compound anthracene, a non-carcinogen, negative. This structural sensitivity should also enable these tests to be employed at the research level to monitor the development of future series or classes of chemicals which possess structural similarities to known carcinogens. However, the ability of a test to distinguish correctly between carcinogens and non-carcinogens of the particular chemical class of compounds being studied will need to be established for each such class.

If the responses of the first two tests are compared it is found that they agree with each other in correctly predicting the activity of 106 of the 120 compounds (88%). In contrast, they both disagree with

long term evidence in only two cases, those of diethylstilboestrol and vinyl chloride (Vinyl chloride produces a reproducible positive result in the Ames test when tested as a gas rather than as a solution in DMSO). The remaining 12 compounds about which the tests disagree represent six established carcinogens and six putative non-carcinogens. Thus, if the results of these two tests are combined and a positive response from either system taken as an indication of activity they register all of the known carcinogens used in this study as positive, with the exceptions of diethylstilboestrol and vinyl chloride, and at the same time generate six false positive results.

Use of short term tests in screening programmes

The main appeal of short term tests is that they seem to offer a method of rapidly searching a group of compounds for potential carcinogens in order that priorities may be set for conventional long term studies. However, the drawback to using even relatively reliable tests for such purposes is that the proportion of positives produced which would be expected to be false may become very large. It is not possible from the present study to accurately estimate this proportion, but the model shown in Table 4 illustrates the nature of the problem which may be encountered. For example, in the model the effect of using a test which was known to

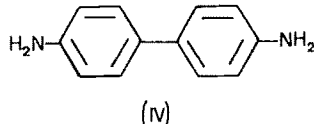
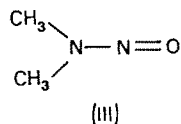
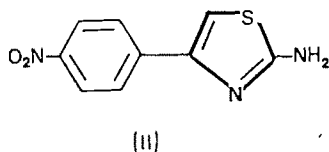
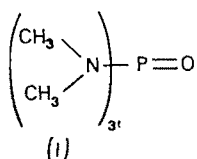
Table 4 Model of the way in which a screening test would operate under various conditions

Test accuracy	Proportion of carcinogens			
	0.1%	1%	5%	50%
99%	11* (91%)†	20 (50%)	59 (16%)	500 (1%)
97%	31 (97%)	39 (75%)	77 (37%)	500 (3%)
95%	51 (98%)	59 (84%)	95 (50%)	500 (5%)
90%	101 (99%)	108 (92%)	140 (68%)	500 (10%)

Positive results generated* for a group of 1,000 randomly chosen chemicals containing various proportions of carcinogens and using tests of various accuracies. The percentage value † refers to the proportion of these positives which would be expected to be false. This model is subject to the following assumptions being made. First, that the test accuracies for carcinogens and non-carcinogens are the same, and second, that results are reproducible and therefore not associated with experimental error. Finally, it has been assumed that when extrapolating data from Table 1 to the model situation of Table 4 the ratio of positive and negative results in the two categories (carcinogens and non-carcinogens) remains constant.

be 90% accurate to screen an arbitrarily selected sample of 1,000 chemicals containing 10 carcinogens (1%) would be to generate 108 positive results of which only nine would be carcinogens. There would, therefore, be only an 8% chance that a compound which was positive in the test would be an animal carcinogen. Conversely, in the present example, the probability that a compound which gave a negative result in the test would be a non-carcinogen would be greater than 99%.

Even if the test systems were to be further improved it is inevitable that there will always remain a finite level of false positives (and false negatives) due to the unavoidable existence of differences in enzyme levels and activities, biological absorption and distribution, DNA repair mechanisms and so on between the test system and the whole animal. Thus, faced with the certainty that any screening programme will generate equivocal positive results it is important to establish in advance how these results would be handled. One approach would be to submit each compound that had been shown to be positive in the screen to conventional animal tests. Alternatively, the future use of all such compounds could be halted. Both of these approaches are drastic and would probably result in unjustifiable economic and social disruption. A more realistic approach to this problem might be to extrapolate existing knowledge of the molecular structures and physicochemical properties of the many established animal carcinogens either to preselect chemicals for submission to the screen or to decide which of the compounds that had shown a positive result in the screen were most worthy of further evaluation. It seems likely that compound preselection would enable a larger proportion of any previously unrecognised environmental carcinogens to be characterised and controlled in the immediate future, and this approach has been developed below.



To act as an effective pre-screen the drawing of structural analogies between untested compounds and established carcinogens must be conducted quite liberally. Thus, it would be necessary to regard all derivatives of hydrazine, all nitrosamines, all compounds containing a polycyclic aromatic nucleus, and so on as candidates for the screen. Moreover, the term 'derivatives' as

used here would have to embrace relatively distant analogues of the various reference carcinogens. For example, any such pre-screen should be so conceived that it would have selected for testing the two recently described carcinogens hexamethylphosphoramide¹⁷ (I) (HMPA) and the aminothiazole¹⁸ (II) based on their structural resemblance to the reference carcinogens dimethylnitrosamine (III) and benzidine (IV) respectively.

Having selected various groups of compounds they should be tested in the presence of the structurally appropriate positive and negative controls. If, for example, compound (II) had been selected for testing it would be prudent to evaluate it in a test system that could, at the same time, be shown to respond correctly to the control pair of benzidine and 3,3',5,5'-tetramethylbenzidine (see Table 3). Greater weight could be given to the result obtained for (II) in such chemical class controlled conditions than had it been generated in isolation.

Two further considerations argue strongly in favour of the use of class-specific as well as test-specific control pairs. The first is that if members of the chosen control pair are correctly distinguished by the test it increases the probability that the physical, chemical and enzymic conditions of the test system have been optimised for the particular class of compound being studied, and the second is that it would be unwise at the present time to assume that any test is equally sensitive to carcinogenicity within all classes of present or future carcinogens. It would, for example, be of little value to monitor analogues of the novel carcinogen HMPA (I) with a test that was incapable of registering HMPA itself as positive.

The main objection to the above approach is that it implies that sufficient is already known of the broad structural features of all established and possible future carcinogens. Such may not be the case and it would therefore be necessary, in addition, to select and screen representatives of new or previously untested chemical classes. If several analogues of such a class were to prove positive in the screen, or if the level of human exposure to such a positive compound was high, the undertaking of conventional long-term testing would be appropriate.

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letters to nature

Continuum variability in Nova Cygni 1975

ONE of the more unusual aspects of Nova Cygni 1975 (V1500 Cyg) has been the presence of nearly periodic (~ 3 h) photometric variations beginning early in the decline (discovered by Tempest¹ on September 9-10, 1975) and lasting up to the present. In spite of the excellent coverage available, the cause of this photometric activity is uncertain. In particular, broadband photometry has been unable to discriminate between continuum and emission line variations, which is necessary because the relative importance of

continuum and line emission has changed as the nova has developed. The relationship of this variation to classical transition phase oscillations in novae is also uncertain. We have obtained very low noise ($s/n \sim 100$) time resolved (~ 11 min per observation, extending over 87 min) 0.8-Å resolution scans of the [OIII] $\lambda\lambda$ 4959, 5,007-Å complex in Nova Cygni starting at 0943 UT on August 21, 1976, using a multichannel Reticon (Vogt and Tull, in preparation) at the coude focus of the 2.7-m telescope at the McDonald Observatory. The scans, which are shown in Fig. 1, establish the stability (profile variations $< 5\%$) of this feature. We

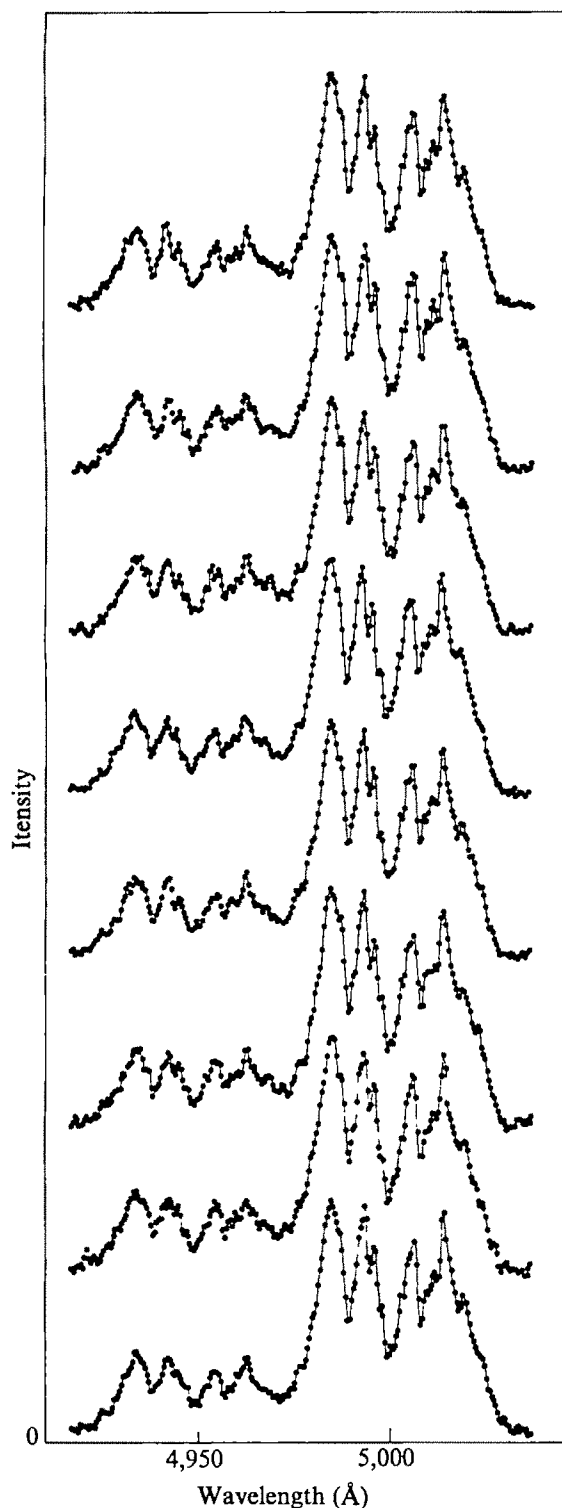


Fig. 1 Scans of the [OIII] 4959, 5,007-Å emission feature in Nova Cygni made on August 21, 1976 with a Reticon at the coude focus of the McDonald Observatory 2.7-m telescope. The scans, from top to bottom, ended at 0946, 0959, 1011, 1022, 1035, 1045, 1056 and 1107 (UT)

infer the constancy of the [OIII] $\lambda\lambda$ 4959, 5,007-Å emission feature and further infer the constancy of the other forbidden lines.

The observations being spectroscopic do not exclude the possibility that the strength of the [OIII] emission feature as a whole is varying. A 3.2-h variation of the emission feature as a whole is very unlikely, however, because of the size of the nova envelope, which at the time of observation was at least 2 light days across.

The emission from a line excited by collisions, such as the [OIII] line, is proportional to $N_e N_A$, where N_e is the electron density and N_A is the density of the atom under consideration. The Balmer lines are formed by capture-cascade whose emission is proportional to $N_e N_{H^+} \sim N_e^2$ where N_{H^+} is the proton density, and we have assumed that hydrogen is the dominant electron donor. Both the collisionally excited and the capture-cascade lines are thus sensitive to the density, and so we may infer that the Balmer series is also constant at present.

Thus only the continuum remains as a possible source of the variation, in spite of the fact that it is a minor contributor to the flux in the Johnson V filter. To check this we undertook two nights of conventional differential photometry using a pulse-counting RCA 8850 (bi-alkali photocathode) on the McDonald 76-cm telescope. BD + 47 3348 was the comparison star. A Stromgren y filter, whose flux is dominated by the continuum, was used together with a Crawford H β wide filter, whose flux is mainly from [OIII] $\lambda\lambda$ 4959, 5,007 Å and H β . The results are shown in Fig. 2 which demonstrates that the continuum is highly variable while the total flux in [OIII] and H β is nearly constant. The small variations in the H β wide filter are consistent with an origin in the continuum. We see that the 3.2-h variability of the nova originates in the continuum.

The contribution of the emission lines to the total flux in each of the broad band filters U, B, V and R varies considerably from one filter to another. Figure 3 shows a low resolution (4 Å) scan of the spectrum of the nova made on August 21, 1976, which has been corrected for a mean instrumental response function, and the UBV system functions given in Johnson². In the U filter the emission lines and continuum make comparable contributions to the total flux. The flux in the R filter, which has peak transmission at 7,000 Å (not shown in Fig. 3), is dominated by the H α , [N II] 6,548, 6,583 Å emission feature. We expect the broadband variability of the nova to be most marked in U and almost absent in R.

It is clear that interpretation of the broadband colours of the nova must allow for the constant emission lines. We cannot be sure how long the emission lines have been constant. A light travel time argument suggests that they must have settled down about the end of September 1975—a month after the nova outburst, when the nova envelope would have been at least 4 light hours across. Jeffers and Weller³ and Campbell⁴ report that in September 1975 the emission line strengths were varying so that during the first month or so after outburst it seems likely that both the continuum and emission lines were varying.

The most fruitful photometric observations of the nova should use filters chosen to exclude the constant emission lines and concentrate on the variable continuum. The Stromgren y filter is an example. Low resolution scans of the nova made by one of us

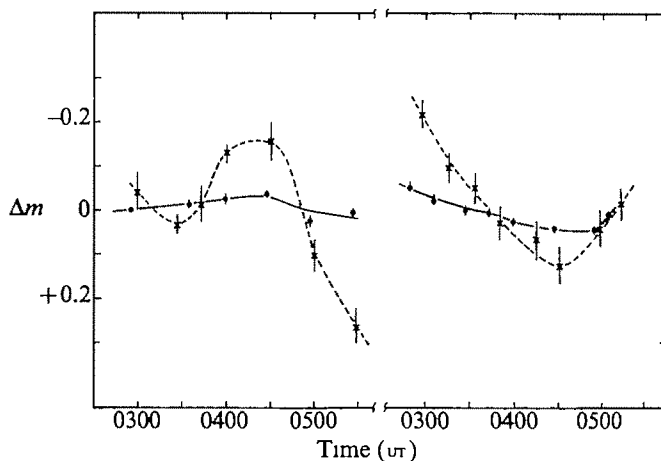


Fig. 2 Photometric observations of Nova Cygni made with a Stromgren y filter (---) and a Crawford H β wide filter (—). The flux in the y filter is mainly from the continuum, in the H β wide filter it is mainly from the [OIII] 4959, 5,007-Å and H β emission lines. The left-hand curves are for August 22, 1976 and the right-hand curves for August 23, 1976.

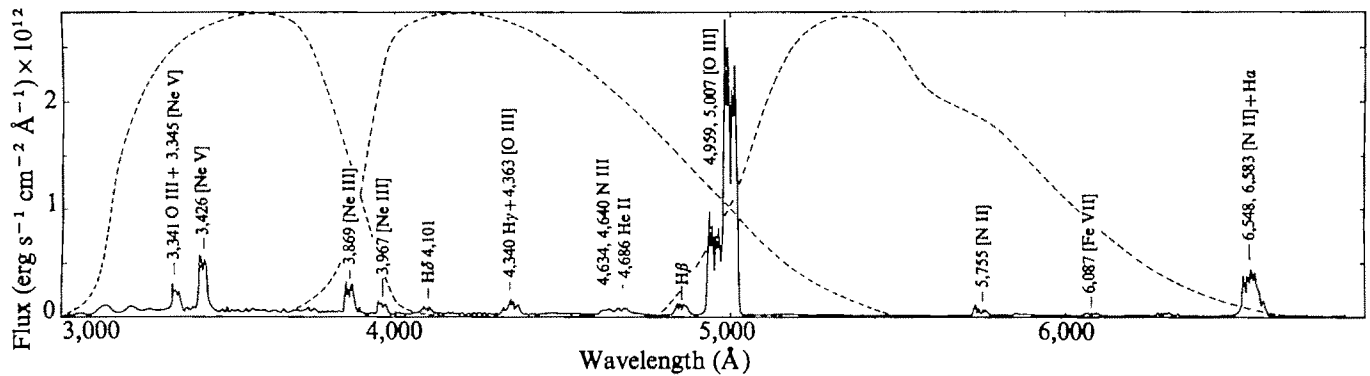


Fig. 3 Spectrum of Nova Cygni obtained on August 21, 1976 with the McDonald Observatory 2.7-m telescope. The absolute calibration (uncorrected for interstellar reddening) was obtained from our continuum observations and is uncertain by 10% because of the continuum variation. The response functions of the Johnson UBV filters are shown.

(J.W.) at regular intervals since its outburst show that since June 1976 there have been no major changes of the relative strengths of the emission lines. Thus, for the present, the scan of the nova spectrum in Fig. 3 should be a good guide for selecting other filters for observing the nova continuum.

We conclude that the continuum variations must originate in the central object rather than in localised regions of the envelope because of the stability of the emission line profiles and light travel time arguments. The importance of further continuum observations cannot be overstated. The object is presently > 7 mag above minimum light in the continuum, indicating that surface activity has not ceased. Further observations aimed at measuring the colour over the phase and determining accurate periods are essential to find out more about the nature of the variation and the central object.

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Spectral analysis and the astronomical theory of climatic change

THE astronomical theory¹ attributes long term changes in climate to changes in the Earth's orbital geometry. This theory has become increasingly accepted in recent years. To this acceptance, workers associated with the CLIMAP project have added exceptionally strong support² using spectral analysis of deep-sea core data. They have linked periodicities in the Earth's orbital parameters with periodicities in deep-sea core parameters related to sea-surface temperature, ice volume and oceanic salinity. Their argument rests on the assumption of a linear response between input (radiation changes caused by changes in orbital geometry) and output (the climate-dependent parameters mentioned above). At the same time they have attributed the anomalously high variance associated with the longest periodicity ($\sim 100,000$ yr) to a nonlinear response. There is no contradiction here because the response function, the climate 'black box', most probably has both linear and nonlinear components. I examine here the question of nonlinear response in more detail.

The input spectra given by Hays *et al.*² show strong peaks at periods of $\sim 41,000$ yr (associated with obliquity), and 23,000 and 19,000 yr (associated with precession). There is also a fundamental periodicity of $\sim 100,000$ yr associated with eccentricity, but this does not appear in the spectral record because eccentricity acts mainly to modulate the amplitude of the precession 'cycle'. The output spectra of Hays *et al.*² show strong peaks at $\sim 100,000$, 43,000 and 24,000 yr. There is also a peak at 19,000 yr in the $\delta^{18}\text{O}$ record (related to ice-volume changes). Most of the variance is contained in the 100,000-yr peak (~ 50 per cent), contrasting strongly with the lack of power at this period in the input signal. Hays *et al.*² convincingly link the 43,000- and 24,000-yr periodicities with obliquity and precession respectively.

Consider the effect of a nonlinear response mechanism on a simple input signal and on an amplitude modulated input signal. Firstly, as Gray³ has shown in a spectral analysis of precipitation data, an input signal with spectral peaks at frequencies ω_1 and ω_2 can produce an output signal with peaks at frequencies $\omega_1 + \omega_2$ and/or $\omega_1 - \omega_2$. A simple mathematical analysis can show how this can occur. Consider an input $X(t)$

$$X(t) = \sin \omega_1 t + \alpha \sin \omega_2 t \quad (1)$$

which is operated on by a nonlinear response function producing an output $Y(t)$

$$Y(t) = [X(t)]^2 + a[X(t)] \quad (2)$$

Note that $Y(t)$ contains a linear term $a[X(t)]$. Substituting equation (1) into equation (2)

$$Y(t) = a \sin \omega_1 t + \alpha a \sin \omega_2 t - \frac{1}{2} \cos 2\omega_1 t - \frac{\alpha^2}{2} \cos 2\omega_2 t + \alpha \cos(\omega_1 - \omega_2)t - \alpha \cos(\omega_1 + \omega_2)t + \frac{1 + \alpha^2}{2} \quad (3)$$

$Y(t)$ contains power at both composite frequencies $\omega_1 + \omega_2$ and $\omega_1 - \omega_2$. Power at the input frequencies ω_1 and ω_2 is only transmitted through the linear part of the response function.

Secondly, consider an amplitude modulated periodic signal of the form

$$F(t) = (1 + \beta \sin \omega_3 t) \sin \omega_4 t$$

where ω_4 is the frequency of the basic signal and ω_3 is the frequency of amplitude modulation. Spectral analysis of this signal produces a major peak at ω_4 with minor peaks at $\omega_4 \pm \omega_3$, but no peak at the amplitude modulating frequency. If, however, this signal is operated on by a simple nonlinear response function

$$Z(t) = [F(t)]^2$$

the spectrum of the output, $Z(t)$, has a dominant peak at the amplitude modulating frequency (and, additionally, an array of

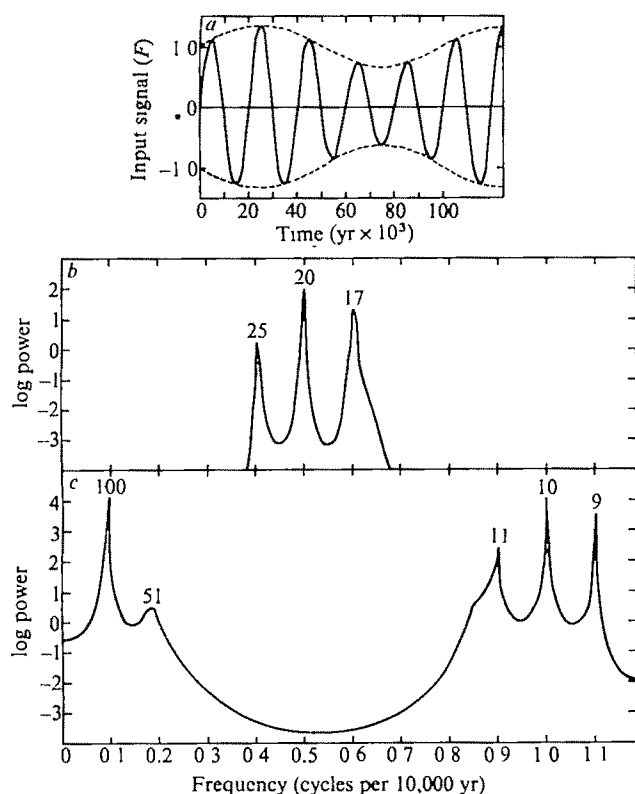


Fig. 1 Amplitude-modulated sine wave signal (a) and MESA spectra of signal (b) and signal-squared (c). The chosen periods of 20,000 and 100,000 yr approximate those of the orbital parameters. For both spectra the Nyquist frequency is 2.5 cycles/10,000 yr (4,000-yr period). A minor peak at a frequency of 1.2 cycles/10,000 yr lies outside the range plotted in the lower spectrum. The numbers above the peaks give periods in thousands of years.

peaks centred around $2\omega_2$). These results are illustrated in Fig. 1 which shows a signal of the type $F(t)$, its Maximum Entropy Spectral Analysis (MESA) spectrum, and the MESA spectrum of $Z(t)=[F(t)]^2$.

The results of Hays *et al.*² can be examined in the light of these simple results, bearing in mind that the real response function must be considerably more complex than the extremely simple ones considered above. The orbital input spectra show a split peak near the precession period of 21,000 yr (Table 4 of Hays *et al.*² gives peaks at periods of 23,100 and 18,800 yr). Nonlinear interaction between these two frequencies could be expected to give an output signal with period 101,000 yr (the $\omega_1 - \omega_2$ term in equation (3)). In addition to this effect, nonlinear response to amplitude modulation of the precession signal will produce a spectral peak at the frequency of amplitude modulation; namely at the eccentricity period of $\sim 100,000$ yr. These two possibilities are closely related if the precession peak splitting is caused by amplitude modulation: the magnitude of the $\omega_1 - \omega_2$ period, 101,000 yr, is remarkably close to the eccentricity period and so admits this possibility.

Either individually, or together, these two mechanisms both lead to strong output power near a period of 100,000 yr, with little or no input power at this period. The data of Hays *et al.*² also show significant power at periods corresponding to precession and obliquity, so the climate response function cannot be wholly nonlinear. The indication is that the response function has both linear and nonlinear parts of approximately equal importance.

This analysis raises two further questions, why is the response nonlinear and why is it that other nonlinear interaction frequencies (such as the $\omega_1 + \omega_2$ term in equation (3)) are not evident in the deep-sea core record? Hays *et al.*² suggest that nonlinearity may arise from a difference in response times for ice build up and decay at the start and end of glacial periods. An alternative answer lies in the albedo-radiation input feedback

mechanism. Climatic sensitivity to energy inputs can be examined usefully using the radiation-balance equation⁴

$$\sigma T^4 = \frac{S}{4}(1-A)$$

where σ is the Stefan-Boltzmann constant, S is the solar constant, A is the global-mean albedo and T is the radiative-equilibrium temperature of the Earth. In terms of perturbations from an equilibrium temperature T_0 at S_0 and σ_0 one obtains

$$T - T_0 \approx \frac{T_0}{4} \left(\frac{\delta S}{S_0} - \frac{\delta A}{1-A_0} \right)$$

where $\delta S = S - S_0$ and $\delta A = A - A_0$. Any feedback mechanism behaving like

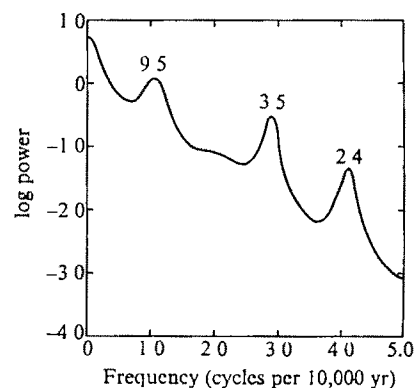
$$\delta A = C_1 \delta S + C_2 (\delta S)^2$$

will yield the form of nonlinear response function considered earlier. There is little doubt that changes in albedo will occur if S is changed (one argument proposed is that lower S produces lower temperatures, more snow cover, and lower global mean albedo), and it seems certain that the albedo-solar constant (or albedo-temperature) feedback will not be linear.

This argument primarily illustrates how feedback may be related to, or cause, nonlinear behaviour of the climate response function. Many feedback mechanisms have been proposed, and most probably more than one operates at any one time. The complexity of the situation can be gauged by the fact that both positive and negative feedbacks between T and A (or between S and A) have been proposed. Deterministic climate models attempt to include important feedback effects, and a crucial test for such models is that they should produce realistic output spectra from correct orbital input data. Since many models are quasi-linear, nonlinearity must be 'built in' through feedback mechanisms.

The absence of other nonlinear interaction frequencies in the deep-sea core spectra may be a result of bioturbation or of the complexity of the climate response function compared with the simple models used here. Furthermore, the response function will differ for each output variable considered, and one need not expect different output variables to show identical spectra (as is indeed the case). One might expect, therefore, that some climate-dependent variables would show other nonlinear interaction frequencies. The $\omega_1 + \omega_2$ frequency corresponding to the 23,100- and 18,800-yr precession peaks gives a period of 10,400 yr. A similar periodicity arises from the amplitude modulation effect (see Fig. 1). This period occurs in the ^{14}C dating anomaly, but the link between this and climate is rather indirect. A period of

Fig. 2 MESA spectrum of oxygen isotope data from Iowa speleothems. The spectrum is a low-resolution spectrum (11 lags, 78 data points), and the data were neither detrended nor prewhitened. The Nyquist frequency is 5 cycles/10,000 yr. Numbers above peaks give periods in thousands of years.



~10,000yr is also evident in the oxygen isotope record from Iowa speleothems⁵. Such isotopic changes have been directly linked to climatic change by a number of workers⁶⁻⁸. A MESA spectrum of Iowa speleothem data spanning the period 6,000–83,000 b.p. (Fig. 2, data supplied by Harmon (personal communication)) has peaks at periods of 2,400, 3,500 and 9,500yr. The peak at ~2,400yr is interesting since a similar period has been detected in the isotopic variations of the Camp Century ice core⁹, and in the frequency of periods of glacial activity in the Holocene¹⁰⁻¹². The dominant peak (significance of MESA spectral peaks being proportional to the area under the peak) is, however, at 9,500yr which may correspond to the $\omega_1 + \omega_2$ nonlinear interaction discussed above.

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Upper-atmosphere zonal winds from satellite orbit analysis

By analysing the changes in the orbital inclination i of a suitable satellite, it is possible to determine zonal (west-to-east) winds in the Earth's atmosphere at heights near that of the satellite's perigee. These values of wind speed are usually averaged over latitudes between 0 and about $0.5i$; often the values are also averaged in local time, although some recent results range over only a few hours in local time.

We have critically reviewed all such results previously published, in the light of current standards of accuracy: some are accepted, some revised and some rejected. We have also

analysed a number of fresh orbits, giving a total of 44 values of the atmospheric rotation rate Λ (rev d⁻¹) from 35 satellites¹. These values, which apply at heights between 150 and 700 km, have been divided into three categories: (1) averaged in local time; (2) 'evening', defined as 1800–2400 local time; and (3) 'morning', defined as 0400–1200 local time.

The values of Λ are plotted against height in Fig. 1 for the three categories of local time. The evening values obtained from orbits analysed at the Royal Aircraft Establishment (RAE)¹⁻³ are indicated by upward-pointing triangles, the morning values^{1,3,4} by downward-pointing triangles, and the average values^{1,5,6} by black circles, all with unbroken 'error-bars'. We have also utilised results based on balloon satellites by Blum and Schuchardt⁷ and by Slowey⁸, and results using short-lived satellites by Forbes⁹; these are shown with broken lines as 'error bars'.

The solid curve in Fig. 1, through the points averaged over local time, shows that the rotation rate (in rev d⁻¹) increases from near 1.0 at 150 km to 1.3 near 350 km, corresponding to an average west-to-east wind of 120 ms⁻¹ at a representative latitude near 30°: the rotation rate then declines to 1.0 at 400 km, and probably to about 0.8 at greater heights. The upper broken curve shows that in the evening the wind is from west to east, increasing from about 50 ms⁻¹ at 150 km height, to a maximum of about 150 ms⁻¹ near 350 km; above that the data are sparse, but a decline to near zero by 600 km seems probable. The lower broken curve shows consistent east-to-west winds in the morning, of magnitude 50–100 ms⁻¹ above 200 km.

It should be emphasised that these results refer to winds averaged over all conditions of solar activity and geomagnetic disturbance. The winds are sampled at latitudes from 0 to 55°, but with a bias towards the lower latitudes. The day-to-day behaviour of the atmosphere may vary widely from these norms: it is the strength—and the weakness—of the satellite orbit analysis that it gives an impression of the average atmosphere. Other techniques, such as radar back-scatter or measuring the motion of vapour trails released from rockets, can give much better resolution in local time and geographical location, but fail to indicate world-wide average values.

Still, it is possible to give some indication of how the wind speed varies with, or depends on, geomagnetic disturbances, solar activity and latitude. The values of Λ in Fig. 1, being averaged, are for quiet-to-medium geomagnetic conditions, but it is well known that winds are usually enhanced at the times of geomagnetic storms, when winds of up to 500 ms⁻¹ have been measured by vapour-trail methods¹⁰, radar back-scatter techniques¹¹ and orbit analysis⁹. We have looked for possible variations of Λ with solar activity and latitude, and

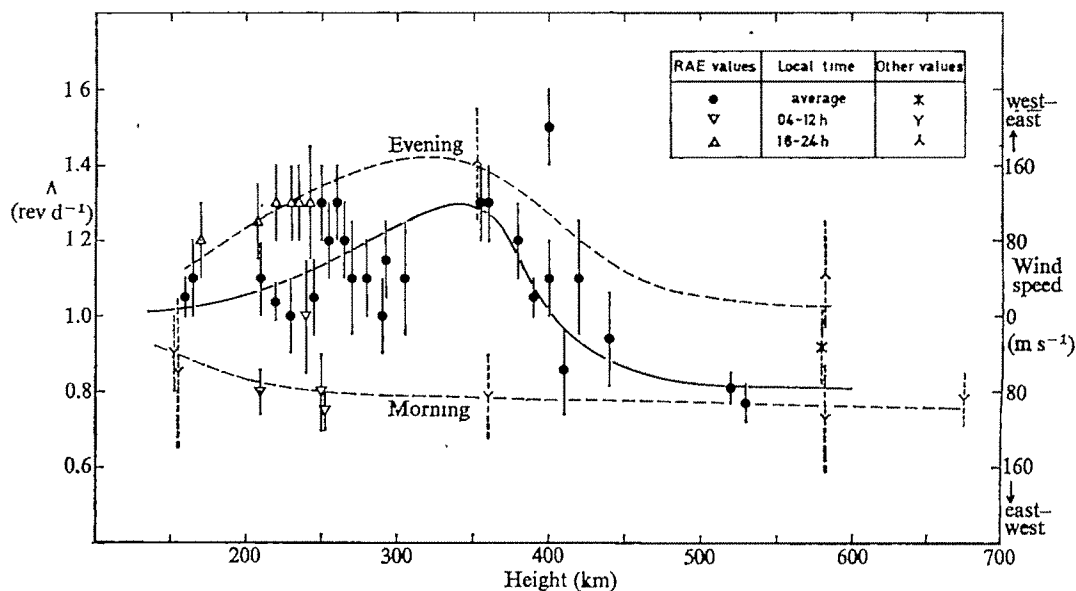


Fig. 1 Atmospheric rotation rate, Λ , against height.

we tentatively conclude from analysis of the values in Fig. 1 that Λ tends to be high when solar activity is low, and that, at heights above 350 km, Λ is larger in near-equatorial latitudes (0–25°) than at higher latitudes (25–50°).

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Radiocarbon dates and deglaciation of Rannoch Moor, Scotland

THE Loch Lomond Readvance affected large areas of the Scottish Highlands, and while the maximal extent of the readvance glaciers is now fairly well established^{1,2}, it is not yet known how long they took to decay³. Three radiocarbon dates have been obtained from a site on Rannoch Moor in the heart of the south-west Grampian Highlands that provide significant new information on the deglacial chronology of Scotland.

The site, Kingshouse 2, is situated in the north-western part of Rannoch Moor (grid ref. NN 282555) at an altitude of about 340 m. It is a small kettle hole within extensive deposits of hummocky moraine that are the product of a stagnating Loch Lomond Readvance ice cap. The subsurface contours of the basin were established by sounding the basin along a grid pattern, and the deepest point located accurately. Successive cores of 5 cm diameter were removed from the deepest point with a piston corer and subsequently analysed for pollen content. Material for radiocarbon assay was taken from four basal cores put down at the corners of a 30 cm square. The full stratigraphic sequence and pollen assemblage zones are shown in Fig. 1.

A date of $9,910 \pm 200$ BP (BIRM-724) was obtained from the peaty-gyttja horizon (lithostratigraphic unit 7) immediately above the contact with the underlying minerogenic sediments of lithostratigraphic unit 6. At that level, the pollen spectrum is characterised by very high percentages of *Juniperus* (over 75% total land pollen), with significant values for *Empetrum* and *Salix Betula* counts are initially low, but there is a marked upward trend in the birch curve immediately above the dated horizon.

Lithostratigraphic unit 5 consists of fine sands with abundant remains of the moss *Racomitrium lanuginosum*, fragments of which provided a date of $10,290 \pm 180$ BP (BIRM-722). Immediately below the moss horizon, a thin layer of greenish-brown gyttja (lithostratigraphic unit 4) was discovered within the predominantly minerogenic sediments, and that organic layer yielded a date of $10,520 \pm 330$ BP (BIRM-723). A single pollen count was obtained from the organic deposit, the spectrum being dominated by *Empetrum* and *Juniperus*. The pollen data and the inwashed moss fragments suggest affinities with the *Racomitretum-Empetretum* and *Juniperetum nanae* associations which are common in parts of the western and northern Highlands today⁴. The former association occurs on block scree or bedrock with undeveloped soils, while the *Juniperetum nanae* represents a transitional association between

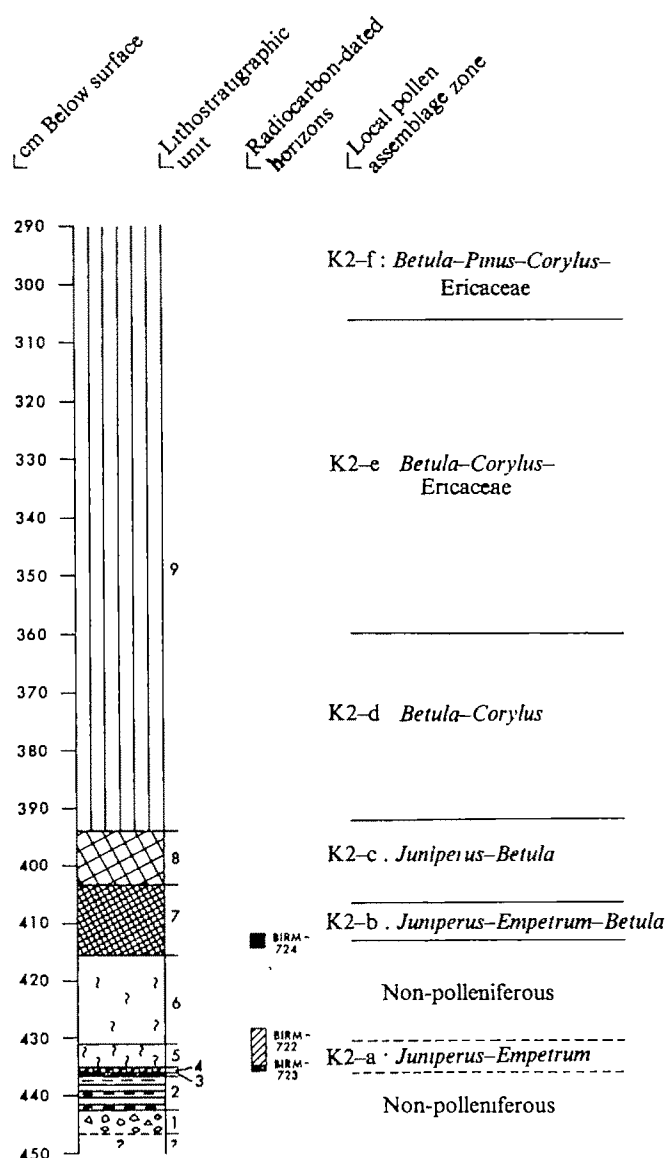


Fig. 1 Lithostratigraphy and biostratigraphy at deepest point of Kingshouse 2 kettle hole. 1, Gravel and coarse sand; 2, laminated silt and fine sand; 3, clay; 4, gyttja; 5, fine sand with abundant moss remains; 6, fine-medium sand with occasional moss fragments; 7, greenish-brown peaty gyttja; 8, light brown gyttja; 9, black telmatic peat with occasional fine roots.

subalpine scrub and low-alpine dwarf shrub heath. The evidence therefore implies that juniper and *Empetrum* heaths became established fairly soon after the deglaciation of Rannoch Moor while soils were still relatively immature, these heath communities being interspersed with patches of bare ground or ground covered by a grass or moss carpet.

The basal rhythmite sequence (lithostratigraphic unit 2) reflects variable conditions of sedimentation within the former lake basin. A maximum of 15 paired laminations was counted for an individual core, although whether these represent true varves is not certain. The lens of gyttja which yielded a pollen

Table 1 Kingshouse 2 radiocarbon dates

Laboratory reference	BIRM-723	BIRM-722	BIRM-724
Site reference	K2-436	K2-433	K2-410
Material	Gyttja	moss remains	Gyttja/lake mud
Weight (g)	54.95	12.40	110.83
Years BP	$10,520 \pm 330$	$10,290 \pm 180$	$9,910 \pm 200$
$\delta^{13}C_{PDB}(\text{‰})$	-22.37	-22.45	-23.30

assemblage dominated by *Empetrum* and *Juniperus* indicates an early period of relative soil stability, but as the organic horizon is very thin and is overlain by more minerogenic sediments, it would seem that that period of stable soil conditions was short-lived. Since *Racomitrium lanuginosum* is a terrestrial moss species^{5,6}, and is therefore unlikely to have been growing around the basin littoral, the abundant fragments of the moss contained in the sands implies that the sediments are the product of increased surface erosion around the basin catchment. This in turn suggests that Rannoch Moor experienced marginal climatic conditions at that time.

The radiocarbon dates from Kingshouse 2 (Table 1) therefore relate to a very early stage in the evolution of the vegetation pattern after the severe climatic regime of the Loch Lomond Stadial, but before milder environmental conditions had become firmly established. In assessing the reliability of the dates, a number of points must be considered. First, there is a fairly large standard error associated with each of the age determinations, due mainly to the small amounts of material available for dating purposes. Secondly, whenever gyttja is used as a dating medium, there is always the risk of hard water error⁷, and while there are no outcrops of calcareous rock within the study area, the possibility cannot be excluded that small amounts of inert carbon may have become incorporated into the drift material from the complex metamorphic rocks that surround the Rannoch basin. Moreover, the small quantities of gyttja material used for dating and the nature of the gyttja itself precluded the use of NaOH in the pretreatment.

Conversely, the age determinations were made on both terrestrial moss and limnic sediments, the dates are internally consistent, and in no case does the $\delta^{13}\text{C}_{\text{PDB}}$ value lie outside the 'normal' range observed for wood, peat, gyttja and charcoal from European localities, that is, $\delta^{13}\text{C}_{\text{PDB}} = -25 \pm 5 \text{‰}$ (Table 1). Also checks have been run on all three dated samples. It was suspected that in the first preparation of BIRM-724 electron capture by electronegative impurities may have led to a slight diminution of the sample activity, and hence a duplicate preparation was undertaken. This second measurement of $9,910 \pm 200$ is preferred. A cross check was also made on BIRM-722, but in this case the same gas sample was counted twice. The two results are not significantly different at the 95% level (2σ), and thus a mean date of $10,290 \pm 180$ BP has been adopted. Finally, BIRM-723 was calculated by using two different values for the modern standard, but as the difference between the two assays was found to be minimal, the date of $10,520 \pm 330$ BP is considered acceptable (R.E.G. Williams, personal communication). Thus, as far as is possible to determine, the dates seem to be reliable within the limitations of the radiocarbon method.

We emphasise that these dates are minimal for deglaciation, for almost 10 cm of minerogenic sediment underlies the lowermost dated horizon, and residual ice may have remained in the basin for hundreds of years after ice had disappeared from the surrounding area⁹. The dates from Kingshouse 2 are the first to be published from the base of a kettle hole within the Loch Lomond Readvance limits, and if correct, provide a minimal date for the disappearance of ice from the vicinity of the site. Taken together, they suggest that Rannoch Moor became ice-free before 10,000 BP, perhaps even before 10,200 BP.

The Kingshouse 2 dates are important in a wider context. Previous studies have shown that the Rannoch basin was one of the major ice accumulation and dispersal centres in Scotland throughout the Pleistocene, and that it performed a similar function during the Loch Lomond Readvance⁸. It seems likely that, because of the altitude and thick ice cover (over 400 m in places), the Rannoch basin would have been one of the last localities in Scotland to be deglaciated following the Loch Lomond Readvance. Hence, if the radiocarbon dates are correct they imply total deglaciation of Scotland some time before 10,000 BP.

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Model of material cycling in a closed ecosystem

ALTHOUGH ecosystem behaviour is ultimately determined by the combination of energy flow through the system and material cycling within it, remarkably little effort has been directed towards elucidating the effects of material cycling on ecosystem stability, noteworthy exceptions being the works of Ulanowicz¹, May², and Dudzik *et al.*³. Ulanowicz¹ and May² studied a model of a linear trophic chain in which both the biomass and energy fluxes between any two levels involved bilinear sums of the biomasses of all the species present and concluded that the system was stable only if the specific energy (that is, energy content per unit biomass) increased on ascending the trophic chain. Although producers are normally found to have a lower specific energy than consumers, there seems to be little or no significant difference between the specific energy of different consumer levels (see, for example, refs 4 and 5). The implication of the Ulanowicz-May model is thus that the stability of a trophic chain is a fragile property dependent on small differences in specific energy between levels. We believe that this result is due to a neglect of the distinctive nature of the dynamics of the decomposition of biological material to inorganic material, a viewpoint consistent with the results of Dudzik *et al.*³ who analysed a number of detailed models of nutrient cycles in both open and closed ecosystems. Here we propose an alternative model of a trophic chain, which suggests that the effect of material cycling is to stabilise the system.

We consider a linear chain of n trophic levels (see Fig. 1) and denote by x_i the number of mol of a given element in the i th trophic level. We follow Ulanowicz and May and model both predation and the uptake of inorganic material by quadratic terms of the Lotka-Volterra type. Our key assumption is that material in the i th level is converted directly to inorganic material at a rate $k_i x_i$. We denote by x_0 the number of mol of the element in the inorganic reservoir. The evolution of the ecosystem is then described by the equations

$$\begin{aligned}\dot{x}_0 &= \sum_{i=1}^n k_i x_i - A_{01} x_0 x_1 \\ \dot{x}_1 &= -k_1 x_1 - A_{10} x_1 x_0 - A_{12} x_1 x_2 \\ &\vdots \\ \dot{x}_n &= -k_n x_n - A_{n,n-1} x_n x_{n-1}\end{aligned}\quad (1)$$

where the principle of conservation of elemental matter guarantees that $A_{ij} = -A_{ji}$ for all i, j . The model is most easily pictured when applied to carbon—the k_i are then respiration rate constants, A_{01} is a rate constant for photosynthesis, and x_0 is the standing crop of carbon in CO_2 . For other elements the terms $k_i x_i$ represent the combined effects of natural death and decomposer action, the implied assumptions being first, that the presence of decomposers does not signi-

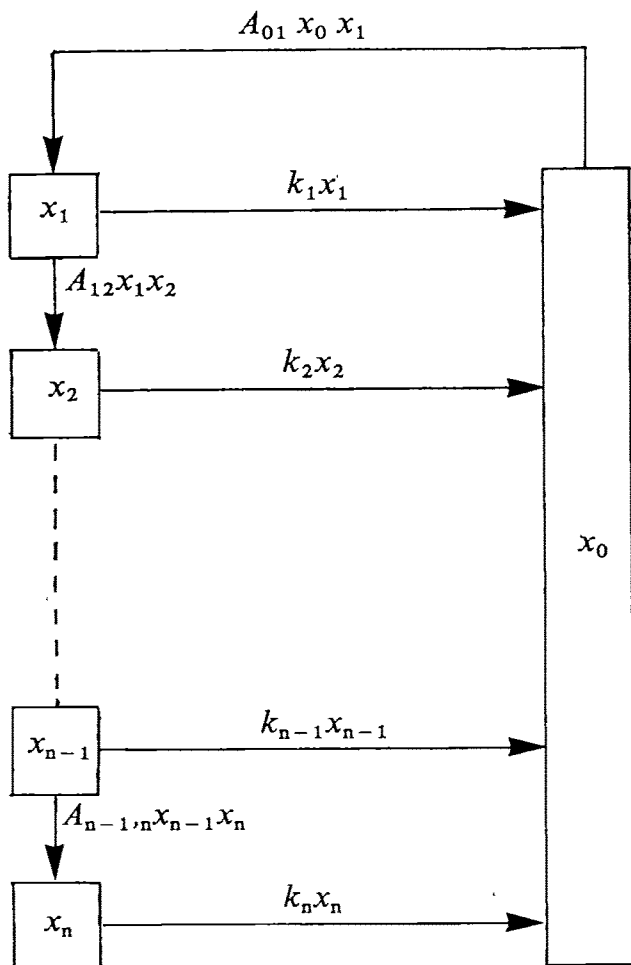


Fig. 1 Standing crops and mass fluxes in the proposed model of an n -level trophic chain.

ificantly influence the rate of provision of material for decomposition, and second, that decomposition is sufficiently fast for us to neglect the time delays in the decomposition process. This latter assumption seems reasonable in tropical and subtropical regions, but is dubious in boreal systems, where decomposition times are typically years (see ref. 6).

Provided only that the above system of equations possesses a steady state in which all of the standing crops are positive, the

system is always stable. We will now outline a proof of this result.

Routine but tedious neighbourhood stability analysis⁷ reveals that the system is stable if the $n \times n$ matrix B_n is stable (that is, if all its eigenvalues have negative real parts) where

$$B_n = \begin{bmatrix} -S_n & -S_n - t_{n-1} & -S_n & -S_n & \dots & \dots \\ S_{n-1} & 0 & -t_{n-2} & 0 & \dots & \dots \\ 0 & S_{n-2} & 0 & -t_{n-3} & \dots & \dots \\ \vdots & \vdots & \vdots & \vdots & \ddots & \vdots \\ \vdots & \vdots & \vdots & \vdots & \vdots & \ddots \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \end{bmatrix} \quad (2)$$

$$\begin{aligned} \text{with } S_i &= -A_{n-i+1, n-i} Q_{n-i+1}, \quad i = 1, 2, \dots, n \\ t_i &= A_{n-i, n-i+1} Q_{n-i}, \quad i = 1, 2, \dots, (n-1) \\ \text{and } Q_i &= \text{Steady-state value of } x_i \end{aligned} \quad (3)$$

By a succession of column operations on the matrix $B_n - \lambda I$ (where I is the unit matrix) we find that

$$\det(B_n - \lambda I) = -S_n \det(B_{n-1} - \lambda I) + \det(A_n - \lambda I) \quad (4)$$

where A_n is the matrix obtained by setting $S_n = 0$ in the matrix B_n . The proof of stability now proceeds by induction. If the matrix B_{n-1} is stable we can use equation (4) to show that it is impossible for B_n to have any pure imaginary eigenvalues. Since the eigenvalues of a matrix depend continuously on the matrix elements, it follows that B_n is either always stable or always unstable. But for one particular choice of model parameters ($k_i = 1$ for all i) we can prove directly that B_n is stable by noting that if Q_i the steady-state value of x_i , is positive then the function

$$M(t) = \sum_{i=0}^n Q_i (x_i - Q_i)^2 \quad (5)$$

is a Liapunov function in some neighbourhood of the steady state. Thus if B_{n-1} is stable so is B_n . For $n = 1$ stability is obvious and so for any n the stability of B_n , and thus of the model, follows by induction.

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Anaerobiosis of fluid mud

OBSERVATIONS in estuaries have reported the existence of ephemeral deposits of semi-fluid mud^{1,2} extending several metres from the bottom. These muds differ from 'mud' as normally understood in that although they form definite boundaries with the overlying water mass they have a lower solid content and settle, if at all, only very slowly. They are also reported to have only 4–400 mg ml⁻¹ suspended solids compared with an average of 1,300 mg ml⁻¹ for bottom muds³. Fluid muds can be detected only by high frequency (30 kHz) echo sounding techniques and have been found to vanish at certain periods of the tide cycle.

To obtain samples of fluid muds a special open-ended sampler⁴ was used, in conjunction with precautions to minimise microbial contamination. Samples were taken from masses located in the Bristol Channel by echo sounding with a Kelvin Hughes type MS26A echo sounder. The samples were returned to the laboratory for microscopic examination, estimation of bacterial numbers and measurement of the oxygen uptake.

Both wet and stained samples were examined by phase contrast and direct microscopy. Unlike ordinary mud from the Bristol Channel the samples contained little or no crystalline or unstainable material, and resembled a light floccular biological sludge. Organic carbon estimations showed between 2.5% and 7% organic carbon compared with 5% for Thames mud⁵ and ~15% for digested sewage sludge. Bacteriological counts were performed by the pour-plate method⁶ and duplicates incubated at 22 °C in the air, for 48 h and also anaerobically in a McIntosh and Fildes⁷ jar. Aerobic bacterial counts were 10⁷ per 100 ml and anaerobic counts 10⁴ per 100 ml.

Measurements on freshly collected liquid mud using a modified Clarke oxygen electrode⁸ showed the mud to be anaerobic immediately after collection and the rate of oxygen consumption to be 20 nmol ml⁻¹ min⁻¹. No samples of water from above the mud layer have shown dissolved oxygen values of <7 p.p.m. and most are close to saturation. It is reasonable to deduce, therefore, that the mud masses are anaerobic. That they have not been previously recognised as such probably arises from their characteristically 'aerobic' brown colour. It has been shown that proliferation of strictly anaerobic bacteria and the production of H₂S and black colours associated with metallic sulphides usually takes upwards of 5 d to develop subsequent to the onset of anaerobiosis⁹.

The high aerobic, and lower anaerobic counts suggest that these ephemeral muds may not persist long enough to become appreciably reduced before being dispersed in oxygenated waters at spring tides.

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To define true meaning

As any lexicographer knows, the meanings of words are not immediately open to introspection. Their covert nature underlies at least one current controversy. Some theorists propose that meanings are composed of semantic primitives¹, whereas others deny the existence of such entities^{2,3}. We have investigated the matter in an evaluation of the responses of naive speakers as a source of lexicographical information.

According to a recent theory, there are two sorts of semantic primitive⁴. The first sort includes such notions as motion, vision, and possession, which are each the basis of a corresponding semantic "field". The second sort occurs across different semantic fields in a variety of semantic formulae; it includes such notions as action, causation, and intention. For example, the meaning of "x propels y" can be roughly analysed as "x does something that causes y to move", where "move" is the primitive that defines the field of motion, and "does something" and "causes" are ubiquitous primitives which occur in such similar formulae as "x does something that causes z to possess y", that is "x gives y to z", and "x does something that causes y to be visible to z", that is "x shows y to z."

The most elementary constituents of meaning are presumably various ineffable mental operations and states: these are the fundamental "particles" that make up the "atomic" primitives which seldom need to be analysed in the everyday use of language. It should consequently be relatively easy to produce a label for a semantic primitive but relatively difficult to define it. Therefore, the simpler a verb's semantic formula, the harder it should be to define the meaning of the verb. "atoms" should be harder to take apart than "molecules". For example, "move" should be difficult to define because it labels something that is virtually a primitive; "come" should be less difficult to define because its formula at least involves an adverbial modification, "move towards the location of speaker or some other specified location"; "bring" should be still easier to define because it involves the ubiquitous primitive of causation, "cause to come with"; and "chase" should be the easiest of these verbs to define because it introduces the further ubiquitous primitive of intention, "move rapidly after as a result of an intention to catch". We predicted that the order of difficulty from easiest to hardest for the four corresponding generic classes of verbs would be: intentional verbs, causative verbs, adverbially modified verbs, and primitive verbs.

In a preliminary study to test this prediction, eight undergraduate students each attempted to define sixteen words (verbs of the four classes drawn from each of the semantic fields of motion, vision, possession and communication). We tried to choose words with the same frequency of usage, but there was a slight tendency for the intentional verbs to be less commonly used. Half the subjects received one set of sixteen words, and the other half received another set of sixteen words. Each word was presented separately with a sentence exemplifying the particular sense to be defined. The subjects, who were tested individually, were told to imagine that they were defining the words for the benefit of a child or a foreigner with an imperfect grasp of the language. They wrote down their definitions in their own words and in their own time. After they had finished this task, they put the verbs into a rank order corresponding to their subjective impression of the difficulty of defining them.

Something of the flavour of the subjects' definitions is conveyed by the following examples: chase: (1) to follow closely—indication of speed and urgency; (2) he

followed the dog and tried to catch it; (3) to chase is sometimes to follow it with the intention of catching up with it; (4) to pursue in order to catch or overtake. Example (2) is one of the few cases where a subject has ignored the instructions and couched the definition of a word in terms of the sentence illustrating its meaning. Despite the superficial variety of the definitions, an amalgam of them captures the essential meaning of the word. In fact, example (4), which is presented for purposes of comparison, was culled from a recent dictionary compiled with considerable linguistic sophistication¹. As anthropologists have known for some time, naive informants can on occasion yield accurate and relevant lexicographical information.

Some of the definitions consisted of synonyms or words with more specific or recondite senses than the *definiendum*. They could hardly be helpful to children or foreigners. Such definitions were particularly prevalent for primitive verbs, accounting for just under 70% of the responses to them. The prevalence of synonyms in the definition of this class of verb attests to the difficulty experienced in attempting to explain their meaning. In addition, it makes it difficult to include this class when rank ordering the accuracy of each subject's definitions. In contrast, it was a relatively straightforward matter to rank order the accuracy of each subject's definitions for the three remaining classes of verb within a given semantic field. Thus, granted our earlier analyses of "come," "bring" and "chase," the following definitions from one subject are ranked in order of increasing accuracy: (1) "come": "to come to a place or situation means to change the place or situation from a different place or situation to the first mentioned place or situation"; (2) "bring": "when something moves from some place to another and takes something with it"; (3) "chase": "to chase is sometimes to follow it with the intention of catching up with it". The first definition fails to mention either movement or the crucial role of the speaker's location; the second definition mentions movement but is not as helpful as it could be about the causal relation since it resorts to a near synonym, "take"; the third definition is very nearly complete. The mean ranks of the accuracy scores were as follows. adverbially modified verbs, 2.4; causative verbs, 2.8; intentional verbs, 3.1; and the ranks conformed in a highly reliable manner to the predicted trend (Page's $L=106$, $P=0.01$). The subjects' own impressions of the difficulty of the task, as revealed by their rank orders of the verbs, also conformed reliably to the predicted trend. Their mean ranks were as follows: intentional verbs, 5.3; causative verbs, 8.4; adverbially modified verbs, 9.5; and primitive verbs, 10.7 (Page's $L=225.5$, $P < 0.01$). The numbers of words and clauses in a definition were not related to its accuracy or subjective difficulty. However, subjective difficulty was negatively correlated with the accuracy score in a highly reliable way (Page's $L=4375.5$, $P < 0.005$).

The predicted order of difficulty was confirmed in two further studies both of which used the measure of accuracy employed in the original experiment. The first study examined 5 subjects' spontaneously spoken, rather than written, definitions: their accuracy was in accord with the predictions. The second study, carried out by Marla Petal, again replicated the trend using the written definitions of both Japanese and English words. Three English-Japanese bilinguals and four English-speaking mono-linguals served as subjects in this experiment.

Of the twenty subjects so far tested, 11 have an overall trend that conforms exactly to the prediction, 7 have an overall trend more in accord with the prediction than against it, and only two subjects have produced results definitely incompatible with the prediction ($P=0.0003$, Sign

test). We conclude that some common words are, indeed, easier to define than others.

Words that can be adequately captured in a definition ought accordingly to have formalised senses, that is, they should be less likely to take on new meanings than words that are less adequately defined. Consequently, the number of different meanings of the 32 words used in our original study would be expected to increase as their semantic formulae became more simple. We tested this prediction by recourse to the dictionary². The mean frequencies of separate numbered meanings in the dictionary³ of the 32 words used in our first study were as follows. intentional verbs, 5.4; causal verbs, 8.8; adverbially modified verbs, 10.3; and primitive verbs, 14.8 (a trend that just fails to reach significance, Page's $L=212.5$, $P>0.05$). This phenomenon and the results of the experimental studies can be explained by postulating the existence of semantic primitives that are easy to label but hard to define. We do not wish to imply that primitives are necessarily retrieved in every linguistic task—a listener may well understand a sentence without having to break it down into atoms. However, it seems that primitives are verbalised in a task that requires naive informants to act as lexicographers and to define the true meaning of words.

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Tidal rhythm in a seabird

GUILLEMOTS (*Uria aalge*) breed on cliff ledges in densely packed colonies often numbering many thousands of pairs. About 25% of the British population of these birds nests in Orkney¹, where their proximity to North Sea oil developments makes it important that the numbers breeding are continuously monitored to check for any adverse effects. One of the difficulties in estimating the breeding population is that the numbers present on cliff ledges show considerable variation, particularly before egg-laying². The data presented here were collected on the island of Copinsay, Orkney, between April 20 and May 13, 1976 with a view to determining the reasons for these fluctuations. They demonstrate the clear effect of a tidal rhythm, a phenomenon previously described for birds which feed close inshore^{3,4}, but not apparently for any species which feeds in deeper water, though Tuck⁵ makes passing reference to the possibility of such an effect at some of the guillemot colonies which he studied in Canada.

Data were collected at a study colony of roughly 80 pairs. The number of birds present on the cliff was counted at 0800, 1200, 1600 and 2000 BST on each day. Figure 1 shows the results of these counts over a 16-d period and indicates substantial variance both within days and between counts at the same time on different days. There was, however, no significant tendency for the counts on some days to be higher than those on others ($0.10>P>0.05$, Friedman two-way analysis of variance). As shown in Table

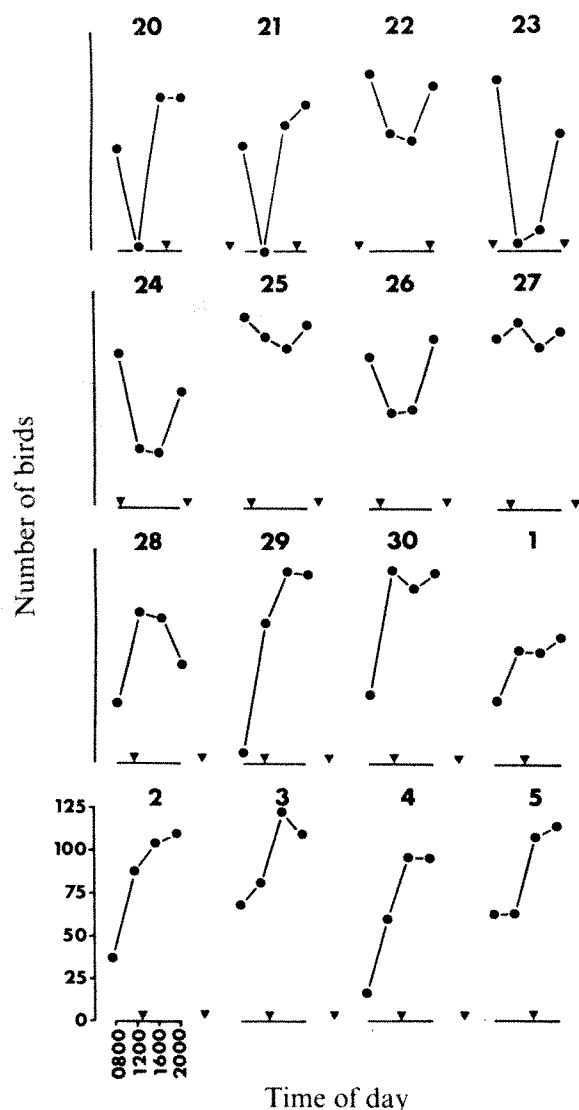


Fig. 1 Number of guillemots present at a study colony at four different times of day on 16 successive days from April 20 to May 5, 1976. Arrows indicate times of high tides.

1, two main factors were found to contribute to the variance between counts: a tendency for numbers to increase during the day and a tendency for them to be highest at around high tide. This tidal effect can be seen in Fig. 1. Initially, the main rise in numbers during the day is between 1200 and 1600 h; on subsequent days it becomes progressively later until by May 5 it occurs in the same interval once again. A 16-d period was chosen for analysis because high tide shows a similar shift round the 24 h, being at 1649 on April 20 and 1655 on May 6. The

Table 1 Variations in numbers of guillemots present with time of day and with state of tide

Time of day (BST)	0800	1200	1600	2000	
Mean number present (N)	63.1 (16)	63.6 (16)	81.8 (16)	93.8 (16)	$P < 0.05^*$
Time since high tide (h)	0-3½	3½-6½	6½-9½	9½-13	
Mean number present (N)	91.9 (16)	75.5 (17)	56.1 (19)	84.8 (12)	$P < 0.05^\dagger$

*Friedman two-way analysis of variance.

†Kruskal-Wallis one-way analysis of variance.

data are not therefore biased towards one state of the tide rather than another.

Observations were also made on the number of birds arriving at and departing from the cliff during 36 1-h watches carried out immediately after some of the counts. Of these, 13 watches were discarded because they were preceded or interrupted by disturbance from gulls calling: at this stage of the season this led to departure of many guillemots from the cliff and their gradual return during the subsequent half-hour. Movements during the remaining 23 watches were analysed to examine the influence of time of day; the same watches were combined with six at other times of day to see whether arrivals and departures were influenced by the state of the tide (Table 2). There was no significant difference in the number of birds arriving at the cliff between the four times of day but departures did vary, being greatest during watches starting at 2000. By contrast, departures did not vary with state of tide while arrivals showed significant variation, the greatest numbers returning to the cliff when the tide was flowing.

Table 2 Variations in arrivals and departures of guillemots with time of day and with state of tide (1-h watches).

Time of day (BST)	0800	1200	1600	2000	
Mean arrivals	21.8	18.0	26.4	26.4	n.s.*
Mean departures (N)	16.6 (8)	16.4 (5)	18.2 (5)	47.0 (5)	$P < 0.02^*$
Time since high tide (h)	0-3½	3½-6½	6½-9½	9½-13	
Mean arrivals	14.0	14.8	27.9	29.3	$P < 0.02^*$
Mean departures (N)	14.7 (6)	14.0 (4)	20.3 (11)	33.0 (8)	n.s.*

*Kruskal-Wallis one-way analysis of variance.

These results indicate a strong influence of state of tide on the number of guillemots present on breeding ledges before egg-laying. The most likely reason for this is that food availability varies with the tide, being greatest at or shortly after low tide, so that birds returning to the cliff after feeding do so mainly when the tide is flowing. Observations of birds carrying fish suggest that on Copinsay, as on the Farne islands⁶, guillemots feed mainly on sandeels (*Ammodytes* spp.), which they catch in mid-water some distance off-shore⁷. The pattern of tidal flow round Orkney is complex⁸, with many areas of turbulence at points where two tidal streams meet. Such disturbance, which varies with the tidal cycle, may bring more sandeels towards the surface and thus within the range of a diving guillemot. Strong currents, such as that which flows eastwards through the nearby Pentland Firth when the tide on Copinsay is rising, may also make food more available by moving shoals of fish through the feeding area. No effect of tides could be found when the observations were repeated in June, at a time when sandeels are abundant and fluctuations in their availability are unlikely to be marked.

In addition to the effects of time of day and of state of tide, the weather also affected the number of guillemots present. A negative correlation was found between wind speed and the average daily count during April/May (Spearman rank correlation coefficient, $r_s = -0.458$, $n = 24$, $P < 0.05$). There was too little rainfall in this period for its effect to be assessed, but during June there was a significant tendency for the second of two counts to be lower when rain occurred during the intervening 4 h (Mann-Whitney U test, $P = 0.004$).

Clearly the number of guillemots present on cliff ledges is affected by a wide range of environmental factors and monitoring procedures must be devised to take account of this.

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H-Y antigen and the origin of XY female wood lemmings (*Myopus schisticolor*)

THE wood lemming, *Myopus schisticolor* Liljeborg, is distinguished by an aberrant sex ratio, with a considerable excess of females, and by the fact that some females produce only daughters^{1,2}. Fredga and his associates³ have provided a basis for understanding both these characteristics. They observed that 82 out of 181 female wood lemmings studied (45%) had an XY sex chromosome constitution, with chromosomal G-banding and C-banding patterns indistinguishable from those of XY males. On the other hand, the XY females were anatomically normal and indistinguishable from XX females. Furthermore, meiotic studies³ showed that the germ line in the somatically XY females was XX. The second X chromosome in the germ cells must have arisen by non-disjunction from the single X present in the XY cells. Thus all progeny from such females must have received copies of the same X chromosome. How does one reconcile these observations with the apparent function of the Y chromosome in mammalian sex determination⁴, or with more recent evidence that a particular Y-linked gene which controls presence of H-Y antigen is critical for differentiation of the male gonad? We have approached these questions serologically by typing male and female wood lemmings for expression of H-Y

antigen, and we have found that all female wood lemmings are H-Y⁻.

H-Y antigen is a cell surface component that is present in males of all mammalian species tested, including the human⁵. In intersexual or sex-reversed individuals, testicular differentiation is more closely correlated with presence of H-Y antigen than it is with presence of an intact Y chromosome⁶. Intersexual X(Tfm)/Y mice⁷ and their human counterpart, XY individuals with testicular feminisation syndrome⁸, are H-Y⁺. So are *Sxr*/XX (sex reversed) male mice⁹, as well as human XX males and XX true hermaphrodites¹⁰. It was therefore critical to ascertain whether lack of testicular differentiation in the somatically XY female wood lemming is associated with absence of H-Y antigen. For this study, 12 animals from the colony at Lubeck were shipped to the Memorial Sloan-Kettering Cancer Center. Multiple tissues (brain, liver, and spleen) were taken for H-Y antigen determination, using procedures fully described elsewhere^{6,12}. Lung and heart were cultured for chromosome studies, according to Miller *et al.*¹⁰.

The four males were H-Y⁺ and the eight females were H-Y⁻ (Table 1). Two of the females had XY karyotypes indistinguishable from those seen in the two successfully karyotyped males. One of the females was an XY/XX mosaic with almost equal proportions of the two cell types scored in cultured lung fibroblastic cells (Fig. 1). Thus in the wood lemming, males are H-Y⁺ and females are H-Y⁻ irrespective of karyotype. The absence of H-Y antigen in XY or XY/XX mosaic females may be responsible for the absence of testes in these females because H-Y antigen appears to act as a 'cell recognition signal' that is essential for virilisation of the indifferent embryonic gonad^{6,11,12}. But what is responsible for the absence of H-Y antigen in these animals, whose Y chromosome looks perfectly normal?

A mutation at the H-Y locus on the Y chromosome might render the gene product both non-antigenic and incapable of performing its essential function. The original mutation would arise in the male germ line, and thereafter XY females could only arise from XY females. But since the germ line in all XY female wood lemmings tested was XX, the Y chromosome in these females could not be transmitted³, and a mutation at the H-Y locus is thus ruled out.

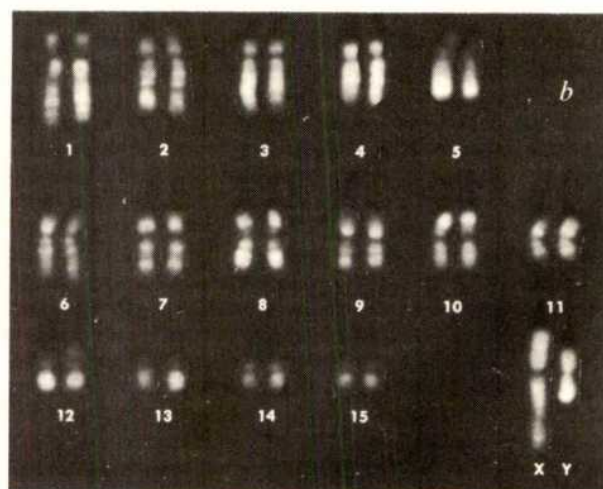
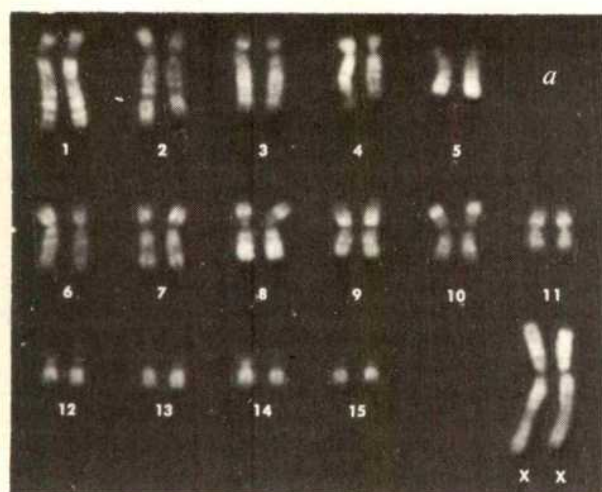


Fig. 1 Quinacrine fluorescence karyotype of a, an XX cell and b, an XY cell from an H-Y antigen negative female wood lemming.

The most likely explanation for the H-Y⁻ phenotype of XY female wood lemmings is that there is an X-linked 'sex reversal' mutation that blocks the expression of H-Y antigen. XY females could arise from matings involving either XX or XY females according to this scheme, which is consistent with the unpublished observations of Frank, Winking and Fredga. Furthermore, loss of the Y chromosome, and non-disjunction of the X to produce an XX germ line in somatically XY females³ would provide a

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Table 1 Sex, karyotype and H-Y antigenic status			
Animal no.	Sex	Karyotype	H-Y Antigen
1645	♂	XY	+
1997	♂	XY	+
1567	♂	?	+
1798	♂	?	+
2047	♂	XY	-
2308	♂	XY	-
2088	♂	XY/XX	-
2140	♂	?	-
2259	♂	?	-
2397	♂	?	-
2407	♂	?	-
2258	♀	XX	-

Tests for H-Y antigen were performed according to procedures fully described elsewhere^{6,12}. Briefly, mouse H-Y antisera were selected, pooled, and the pools divided into three parts. One part was unabsorbed and the other two parts were absorbed with cells from female and male wood lemmings, respectively. Positive absorption (indicating presence of H-Y antigen on the absorbing cells) was manifested as a decrease in reactivity of absorbed sera (decrease in number of mouse sperm killed in the cytotoxicity test and fall in the number of mouse sperm labelled in the mixed haemadsorption-hybrid antibody test; see ref. 12).

basis for obtaining all-female litters, since all the XY progeny would receive an X chromosome with the mutant gene. Our finding that one female was an XY/XX mosaic and the not infrequent occurrence of other sex chromosomal anomalies (XO, XYY, XXY) in the laboratory bred stock of *Myopus* suggest that the mechanism by which the Y chromosome is lost and the X chromosome constitution doubled may represent a more general phenomenon not always restricted to the germ line. And this raises the question of what triggers these events and the range of variability in its timing. For example, could some XY females have XY germ cells? If so, then XY females could occasionally produce male progeny even though they usually do not.

Whether XY wood lemmings develop as males or females seems to depend on whether or not H-Y antigen is expressed on their cells, and this in turn depends upon an X-linked gene as well as the Y-linked H-Y locus itself. In view of the evolutionary conservation of the mammalian X chromosome⁴, a similar X-linked gene may be present in other mammals as well. Indeed the familial occurrence of XY females with gonadal dysgenesis in the human species may be due to a mutation at the same gene locus. Because they have streak gonads and are therefore sterile, affected individuals can arise only from matings involving normal XX females.

The pattern of inheritance is that of an X-linked recessive or autosomal dominant but sex-limited trait, with a single copy of the mutant gene sufficient to suppress testicular differentiation¹³⁻¹⁵. By analogy with the wood lemming, and in view of the critical role of H-Y antigen in directing testicular differentiation, we would expect the XY females in these families to be H-Y⁻. On the other hand, sporadic cases could arise as a result of a spontaneous mutation involving either the same X-linked locus or the Y-linked H-Y determinant.

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T-cell idiotypes are linked to immunoglobulin heavy chain genes

FOUR gene clusters have products that seem to participate in antigen binding on T or B lymphocytes. Three (the genes coding for the heavy and light chains of immunoglobulin molecules) are known to code for structural elements of B-cell receptors for antigen. There is evidence that a fourth group (the immune response genes associated with the major histocompatibility complex (MHC) locus) generates T-cell recognition structures for

Table 1 No linkage between inheritance of T-cell idiotypes and major histocompatibility locus genes

Animal no.	Sex	Phenotype	Uptake of anti-(Lewis anti-DA) anti-idiotypic (mean c.p.m. \pm s.e. of triplicates)	
1	male	L \times BN	+	6,313 \pm 168
2*	male	L \times BN	—	3,394 \pm 85
3	male	BN	—	2,918 \pm 360
4	male	L \times BN	+	8,216 \pm 253
5	male	L \times BN	+	5,668 \pm 271
6	male	BN	—	2,905 \pm 218
7	male	BN	+	6,251 \pm 97
8	male	L \times BN	—	3,632 \pm 73
9	male	BN	+	6,267 \pm 678
10	male	L \times BN	—	3,643 \pm 212
11	male	BN	—	3,508 \pm 118
12	female	BN	—	3,163 \pm 148
13	female	BN	—	2,950 \pm 74
14	female	L \times BN	+	6,050 \pm 162
15	female	BN	+	5,949 \pm 216
16	female	L \times BN	—	3,183 \pm 319
17	female	L \times BN	+	6,247 \pm 59
18	female	L \times BN	+	5,911 \pm 416
19	female	L \times BN	—	3,175 \pm 43
20	female	BN	—	2,859 \pm 59
21	female	L \times BN	—	3,459 \pm 338
22	female	L \times BN	—	3,490 \pm 179
23	female	BN	+	8,049 \pm 856
Controls				
Lewis			+	9,361 \pm 905
BN			—	3,151 \pm 51

* + Denotes specific uptake, — denotes no specific uptake.

Individual offspring from mating (L \times BN) F_1 hybrids with BN rats were typed for Ag-B phenotype using Lewis anti-BN and BN anti-Lewis alloantisera and 125 I-labelled protein A as a marker². For determination of anti-idiotypic uptake 1×10^7 purified T lymphocytes were incubated with anti-(Lewis anti-DA) antiserum followed by 125 I-labelled protein A as a marker².

antigen¹. No evidence exists that any of these four gene groups are linked. In a study of the T-cell receptors for antigen we used anti-idiotypic antibodies against specific T-cell receptors²: T and B cells specific for the same antigen were shown to express similar idiotypes². Analogous findings using other systems and approaches have been reported^{3,4}. Together these results strongly suggest that antigen-binding receptors on T and B lymphocytes at least in part make use of the same genetic material. Accordingly, T-cell receptors should at least partly be coded for by genes linked to "conventional" immunoglobulin gene groups. We prove here that idiotype T-cell receptors with specificity for MHC antigens in the rat are coded for by genes linked to the heavy chain immunoglobulin genes.

Our experimental setup consists of inbred strains of rats (Lewis, BN or DA) which differ as to the rat MHC genes, the Ag-B or H-1 locus. Lewis and DA also differ with regard to allotypes of the IgA class and of the κ light chains⁵⁻⁷. Lewis T cells with immune

reactivity against, for example, Da MHC antigens carry distinct idiotypes different from those on Lewis T cells with reactivity against BN antigens². Anti-idiotypic antisera against the Lewis T receptors with specificity for the DA Ag-B antigens were produced by immunisation of (Lewis \times DA) F_1 rats with Lewis T lymphocytes². Anti-allotype sera specific for the Lewis IgA or κ light chain allotypes were prepared as previously described⁵. Using this setup we started to investigate whether the Lewis anti-DA T-cell idiotypes were inherited in a manner indicating linkage to either the MHC locus or the heavy chain or the κ light chain gene clusters.

A direct search for linkage between T-cell idiotypes and MHC locus genes was first carried out. Using the described anti-idiotypic antiserum we analysed the T lymphocytes of individual offspring from mating of (Lewis \times BN) F_1 hybrids with BN rats. Such rats were typed for their respective Ag-B composition and presence or absence of the Lewis anti-DA idiotypes on some of their T lymphocytes. Table 1 shows that the T-cell idiotypes are not linked

Table 2 Linkage between inheritance of T-cell idiotypic and heavy chain allotypes (uptake of anti-idiotypic)

F_2 rat no.	Lewis heavy chain	Lewis light chain	Uptake of radioactivity (mean c.p.m. \pm s.e. of triplicates)	
			Incubated with anti-(Lewis anti-DA) anti-idiotypic	Incubated with (Lewis \times DA) F_1 normal serum ¹
1	—	—	3,813 \pm 113	3,787 \pm 73
2	—	+	4,106 \pm 254	3,925 \pm 48
3	—	+	4,478 \pm 57	3,783 \pm 166
4	+	+	5,816 \pm 344*	4,016 \pm 63
5	+	+	5,675 \pm 146*	3,872 \pm 41
6	+	+	5,824 \pm 34*	3,890 \pm 104
7	+	+	7,218 \pm 386*	3,933 \pm 109
8	+	+	7,395 \pm 199*	3,824 \pm 34
Controls				
Lewis				
1	+	+	8,234 \pm 319*	3,960 \pm 74
2	+	+	8,432 \pm 98*	3,801 \pm 37

*Specific uptake of anti-idiotypic antibodies.

(Lewis \times DA) F_2 rats were typed for Ag-B antigens using Lewis anti-DA and DA anti-Lewis alloantisera and 125 I-labelled protein A as a marker². Only those rats homozygous for the Lewis Ag-B antigens were used. Sera of such rats were tested for presence or absence of the respective allotypes using gel diffusion technique. For determination of anti-idiotypic uptake see Table 1.

to the MHC genes. Roughly half of the rats typing as homozygous for the BN Ag-B and half of those typing as (Lewis × BN) F₁ hybrids were positive in the radioimmunoassay detecting the idiomotype.

In a second series of experiments we looked for linkage between T-cell idiotypes and heavy or κ light chain allotypes. (Lewis × DA) F₂ rats were typed for Ag-B antigens and only those homozygous for the Lewis Ag-B antigens were used for the subsequent tests. Sera from such rats were obtained and tested individually for presence or absence of the respective allotypes using gel diffusion techniques. The rats were then killed and their T lymphocytes from spleen and lymph nodes obtained after filtration through anti-Ig columns². Samples of the T cells were used in a radioimmunoassay with the anti-idiotypic serum to determine whether or not the cells contained the Lewis anti-DA idiotypes². The remaining cells were used in MLC cultures against DA or BN stimulator cells after the responder cells had been treated with anti-idiotypic or normal serum in the presence of complement². Table 2 shows that three out of eight F₂ rats typed as being homozygous for the DA heavy chain genes (they did not express the Lewis IgA allotype in their

T cells by physical and functional criteria in these animals with the appropriate heavy chain allotype, taken together with the findings in the mouse using an entirely different antigenic system, would seem to solidly establish this inheritance pattern of T-cell idiotypes.

Is it possible to reconcile the present findings with the reported antigen-specific T-cell-derived molecules carrying serological markers indicating them to be products of the IR genes^{9,10} mentioned earlier? Could they, for instance, be the very same molecules where one chain carried the idiotypes detected in the present assays, and another chain carried the Ia-associated antigenic markers? Our analyses of the biochemistry of the idiotype T-cell receptors have, unfortunately, so far failed to indicate that this is true. When isolated from T-lymphocyte supernatants or serum our T-cell-derived, antigen-binding and idiotype molecules have a molecular weight of ~ 150,000 (ref. 11). These molecules can be split into two single polypeptide chains still expressing idiotypes and antigen-binding ability with a molecular weight of slightly above 70,000. These single chains can then be further degraded into molecules of a size range from 30,000

Table 3 Linkage between inheritance of T-cell idiomotype and heavy chain allotypes (MLC inhibition)

F ₂ rat no.	Lewis heavy chain	Lewis light chain	MLC inhibition* against DA stimulator cells	MLC inhibition* against BN stimulator cells
1	—	—	0	0
2	—	+	15	0
3	—	+	12	13
4	+	+	71	0
5	+	+	87	0
6	+	+	83	3
7	+	+	74	0
8	+	+	90	10
Controls				
Lewis				
1	+	+	94	0
2	+	+	95	1

*Responder lymphocytes from F₂ rats or normal Lewis control rats were incubated with anti-Lewis anti-DA/anti-idiotypic antiserum and complement before initiation of the MLC². Figures denote % reduction compared to the control treated with (Lewis × DA)F₁ normal serum and complement. For further details see text of Table 2.

sera). Precisely these three rats were also negative with regard to the expression of the Lewis anti-DA T-cell idiotypes, whereas all the other five rats were positive. This thus strongly indicated that the T-cell idiomotype-coding genes are linked to the genes coding for the heavy chains of conventional immunoglobulins.

The inheritance of the Lewis κ light chain allotype did not show a similar pattern *vis-à-vis* the T-cell idiotypes. One of the eight rats typed as homozygous for the DA κ light chain genes and this rat also happened to be in the idiomotype negative group. Two rats, however, were idiomotype negative but Lewis κ light chain allotype positive.

Final proof that the radioimmunoassay did indeed detect the inheritance patterns of functionally relevant T-cell receptors came from the experiments on the inhibition of MLC using anti-idiotypic antisera (see Table 3). Treatment of the individual Lewis spleen cell suspensions with anti-(Lewis anti-DA) idiomotype serum plus complement never caused any reduction in MLC reactivity against BN MHC antigens. However, T cells from five suspensions displayed highly significant reduced activity against DA antigens after such treatment, whereas three suspensions retained normal reactivity against DA. The five with reduced activity were derived from the five F₂ rats which were positive for Lewis heavy chain allotype, whereas the "reduced" suspensions came from the three rats typing as homozygous for the DA heavy chain immunoglobulin genes. Chi-square analysis of data demonstrated the correlations between idiomotype and allotype to be highly significant. It could thus be conclusively shown by two aspects that the inheritance of T-cell idiotypes is linked to heavy-chain allotype expression.

A similar conclusion has been reached using heterologous anti-idiotypic antibodies in the mouse to activate specific helper cells⁸. The present direct demonstration of the inheritance of idiotypes on

to 40,000 (refs 11, 12). Only the latter molecules have sizes resembling those reported for the Ia-associated T-cell factors and our 30,000–40,000-dalton molecules can be shown to be single chains with idiotype determinants (the constitution as to chain type of the Ia-positive antigen-specific T-cell molecules is unknown but Ia antigens in general are two-chain structures¹³). Furthermore, we have so far been unable to detect any additional chain, nor have we found serological markers of Ia type on our idiomotype-positive T-cell molecules. Molecules similar to ours in size and serology have also been isolated from mouse T cells¹⁴ adding further weight to our statements. It would thus seem clear that no simple model exists as yet that can reconcile the reports on specific factors from T cells being coded for by Ir genes linked to MHC genes with the present ones showing linkage of T-cell idiomotype expression to heavy chain immunoglobulin genes.

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Antibody patterns in genetically identical frogs

THE origin of antibody diversity is still unclear. Either the 10^4 to 10^6 different antibody specificities of the vertebrate immune system are completely encoded in germ line genes, or somatic mutations or recombination of a few genes contribute substantially to diversity. Both the large pool of specificities for single antigens and the very heterogeneous specificities among different individuals of inbred mouse strains (which are assumed to be genetically homogeneous) have led to somatic mutation theories. We have studied the immune response in *Xenopus* frogs which can be heterozygous and at the same time genetically identical. If there are many identical antibodies against several antigens in different individuals, somatic changes of genes coding for antibody specificities cannot be important for antibody variability in such animals. Antibodies to two different antigens have been assayed for by two techniques: inactivation of antigen-coupled phages by antibodies including inhibition of inactivation by free antigen; and isoelectric focusing (IEF). The results show low variability of antibody specificity, and often identical patterns among different individuals having identical genetic background.

Crosses of *X. laevis* (L) with *X. gilli* (G) yield female (LG) offspring which lay diploid eggs, the genome of which is

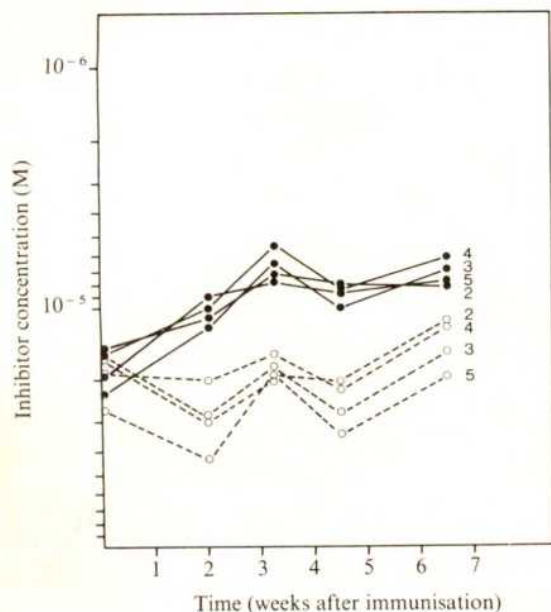


Fig. 1 Inhibition with DNP-lysine (●), and TNP-lysine (○) of the inactivation of DNP-T₄ phage by serum of isogenetic *Xenopus* hybrids immunised with 20 µg DNP-KLH. Difference in concentration of ligand necessary for 50% inhibition as a function of time after injection. Each number corresponds to a single animal.

identical to that of the mothers. The gynogenetic development (that from the female pronucleus alone) of these eggs gives rise to individuals which are genetically identical; that is, they form a clone¹. After a single injection of 20 µg dinitrophenyl-Keyhole limpet haemocyanin (DNP-KLH) in Freund's complete adjuvant into these isogenetic animals, the level and relative affinities of anti-DNP antibodies were measured by the technique of inactivation of DNP derivatised bacteriophage T₄. The kinetics of the response (that is the inactivation constant of the serum as a function of

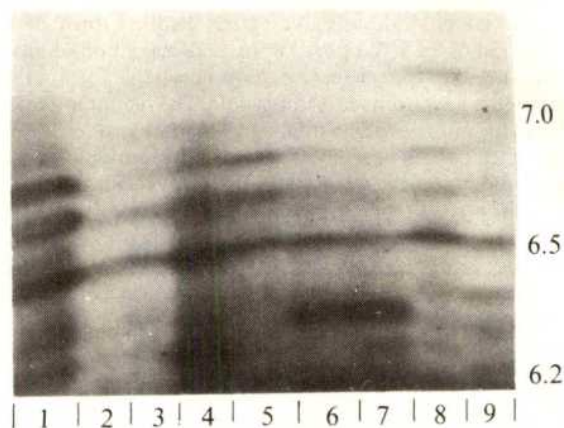


Fig. 2 Anti-DNP sera of 9 individuals of clone LG₁₇, secondary response. The animals were first injected with 20 µg DNP-KLH, and 36 weeks later with 40 µg DNP-KLH. Sera were collected 3 weeks after the reinjection. Individuals 1-5 show identical IEF spectrotypes. Individuals 6-9 which show different clones in addition to a common LG₁₇ spectrotypes had previously rejected a skin graft of *X. laevis*. The primary anti-DNP response after 10 weeks, though weaker, was almost identical to the secondary response in this case.

time) were superimposable for each isogenetic animal from a given clone, whereas for outbred *X. laevis*, the responses differed². The same was true for their discriminatory capacity between DNP and TNP (trinitrophenyl-) groups over a seven-week period, as measured by inhibition of phage inactivation (Fig. 1).

Antibodies titrated by phage inactivation are predominantly IgM³. These antibodies are the first to appear in this response, and they were always present during it.

Three to six weeks after injection of antigen, low molecular weight antibodies to DNP appeared. The patterns of these antibodies as evaluated by IEF^{4,5} revealed restricted heterogeneity and almost identical patterns for different individuals (Fig. 2). Of the five clones studied, three had the same antibody patterns (LG₇, LG₁₅, LG₁₇). These three

Fig. 3 Anti-SRBC sera of individuals of two clones, collected 6 weeks after immunisation with a single dose of 10⁸ SRBC. After IEF the gels were overlaid with SRBC in 0.5% Agarose containing guinea pig complement. Individuals 1-4 belong to clone LG₁₅, from 5-7 to clone LG₁₄. No. 8 is a prebleed.

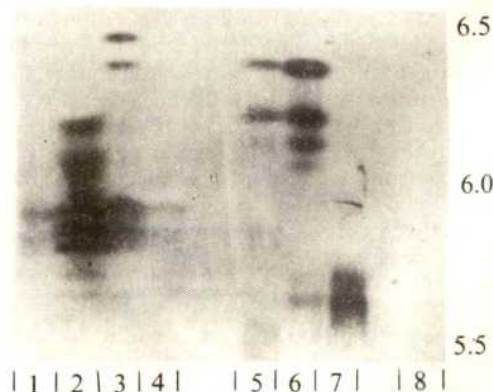
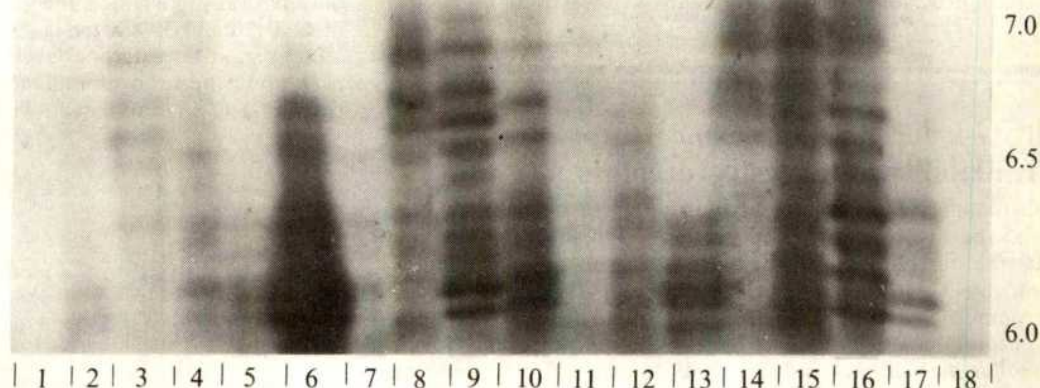


Fig. 4 Anti-DNP sera of 17 outbred individuals of *X. laevis*. The animals were injected with 40 µg DNP-KLH, and 13 weeks later with 40 µg DNP-KLH. Sera were collected 2 weeks after the second injection. Animal 18 was injected with streptococcal carbohydrate.



clones are identical at the major histocompatibility complex (MHC), as determined by the mixed lymphocyte reaction⁶. A fourth clone, LG₅, shares one MHC haplotype with the other three. Some of its members also displayed this IEF pattern of LG₁₅, LG₁₇ and LG₇; other members exhibited different patterns. The two members of a fifth clone (LG₂) tested displayed an identical antibody pattern which was different from that of the other clones. These animals also differed from those in the other clones by at least one MHC haplotype. Some individuals in a clone had rejected a skin graft from a genetically unrelated *X. laevis* donor before they were immunised with DNP-KLH. These individuals partially differed in their antibody patterns (see animal 6 and 7, Fig. 2, and less prominently 8 and 9). Thus, 'environmental' action on the immune system may influence the immune response pattern as evaluated by IEF.

Hapten-binding proteins were detected in one series of experiments by radioactive hapten binding and subsequent autoradiography⁴. In a second series antibodies were identified by complement-dependent lysis of TNP-derived (SRBC)⁵. With this technique, the same results as with radioactive hapten binding were obtained. The immunoglobulin nature of these proteins was demonstrated by the following observations: First, no antibody bands were seen in the pre-immune serum. Second, a pool of sera from animals of one clone was passed over an anti-immunoglobulin adsorbent column and subsequently did not show the bands. When the pooled sera were passed over an anti-µ column, however, the hapten-binding pattern on the IEF gel was present. Third, in the case of the TNP-SRBC detection system, no lysis could be seen without complement.

Given the assumption that identical banding in IEF gels reveals identity in primary antibody structure, the primary anti-DNP response in *Xenopus* seems to be due to the expression of germ line V genes. If somatic diversification had occurred, it occurred non-randomly, or rarely.

Obviously, the structural genes present in the germ line can code for at least some functional antibodies, regardless of any other mechanism for further diversification. Nevertheless, if somatic events are, in fact, necessary for generating antibody diversity, a substantial number of antigen specificities should not be covered by germ line genes. Thus we studied the immune response in the isogenetics to another antigen that displays many different antigenic determinants. The animals received a single dose of 10⁹ packed SRBC. Sera were collected after 6 weeks and run on IEF gels. After the run, SRBC were overlaid on the gel. Following the diffusion of the antibodies out of the gel, specific antibodies were detected by the appearance of lysis of the red cells after the addition of guinea pig complement⁷ (Fig. 3). Addition of anti-Ig antiserum

was not necessary. As with antibodies to DNP, the heterogeneity of antibodies to SRBC was also restricted. The four individuals of the clone LG₁₅ had three bands in common. After a long exposure to complement, two more shared bands appeared (not shown). The IEF patterns of this clone differed from that of the three individuals of clone LG₁₁. Within clone 14, animals 1 and 2 shared two bands, as did animals 2 and 3.

In view of the antigenic heterogeneity of SRBC as compared to DNP, it was surprising that *Xenopus* responded in such a restricted way. Thus the existence of an extra clonotype (individual no. 3 of clone LG₁₅, Fig. 3) was by no means surprising.

The high coincidence of isoelectric focusing suggests germ line genes coding for the primary anti-sheep red blood cell (SRBC) specificities (which are responsible for the complement dependent lysis).

When the animals had been reinjected, the pattern for anti-SRBC antibodies were more heterogeneous. Even though differences between different individuals of the same clone became more pronounced than in the primary response, many bands were still shared by the different individuals.

Although the primary and secondary response of outbred individuals to DNP and SRBC was heterogeneous (though not as heterogeneous as in mouse) and differed much more from one individual to the other than in the case of isogenetic animals, shared bands were detected (Fig. 4).

Inbred mouse strains show great heterogeneity in their IEF patterns to cross-reactive antibodies to DNP and TNP⁸ to NIP (4-hydroxy-5-iodo-3-nitro-phenacetyl)⁹, SRBC⁷, and of antibodies which reconstitute mutants of the enzyme β-galactosidase to wild-type activity¹⁰. Isogenetic *Xenopus*, however, show a high incidence of coincidence in IEF bands of antibodies in their primary anti-DNP and anti-SRBC responses. Although the occurrence of some somatic changes of germ line genes is not excluded, the concept of somatic generation of antibody diversity in *Xenopus* is not necessary in order to interpret these data. While somatic mutation may be the driving force for antibody diversity in mammals, *Xenopus*, a primitive anuran amphibian, might lack an appropriate mutator mechanism.

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hCG-induced decrease in availability of rat testis receptors

EVIDENCE is slowly accumulating to suggest that hormones may be involved in the regulation of their own receptors in the target tissue¹. The present paper reports findings which suggest that treatment with human chorionic gonadotropin (hCG) may influence the availability of LH/hCG receptors in the immature rat testis. These receptors show a similar high affinity and specificity for LH/hCG to those of the adult rat testis^{2–4}.

Human chorionic gonadotropin was used as in other studies^{3–6} because of its ready availability in purified form with high biological and receptor-binding activity. Its effects on testis LH/hCG receptors was studied in three ways. First, the duration of effect of gonadotropin on testis receptors was determined by killing rats at intervals ranging from 6 to 90 h after hCG injection. Second, the dose-response relationship between hCG and receptor availability was examined. Third, the effect of short term exposure to hCG was studied by removing free hCG from circulation, with an excess of antiserum, at 4 and 8 h after injection of hCG, and then killing animals at 20 and 30 h after the hCG injection. To give a measure of the availability of vacant receptors for LH/hCG, the *in vitro* binding of ¹²⁵I-hCG to homogenates of testes from saline-treated controls and hCG-treated animals was compared. The degree of receptor site occupancy was assessed by injecting 0.5 μ Ci ¹²⁵I-hCG into rats with or without 10 IU hCG, and then measuring the concentration of radioactivity in the blood and testes at intervals between 4 and 40 h after injection.

After injection of 10 IU hCG, serum concentrations, measured by radioimmunoassay⁷, declined from 8 to 40 h and were undetectable (<3 mU ml⁻¹) at 70–90 h (Fig. 1). In the saline-treated controls, serum levels of testosterone, measured by radioimmunoassay⁸ after column chromatography⁹, were undetectable (<54 ng per 100 ml) in all but one animal, whereas hCG-treated rats showed elevated testosterone levels for up to 48 h after injection, with highest values at 6–12 h (Fig. 1). The *in vitro* binding of ¹²⁵I-hCG to testis tissue was not significantly reduced (compared with controls) until 8 h after hCG injection (Fig. 1); thereafter, binding declined to 10% of control levels at 30 h after injection, remained at this low level until 48 h, then progressively recovered towards control levels between 70 and 90 h. Varying the dose of hCG injected (0.1–10 IU) had a

dose-related effect on the *in vitro* binding of labelled hCG at 42 h after injection, and as little as 0.01 IU hCG still significantly reduced binding and caused an elevation of testosterone levels (Table 1). Injection of antiserum to hLH only partially prevented the reduction in binding caused by hCG at 20 and 30 h after injection, and did not significantly affect serum testosterone levels (Table 2).

In vivo, the ratio of radioactivity in the testes to that in the blood (T/B) increased significantly ($P < 0.05$) from 4 to

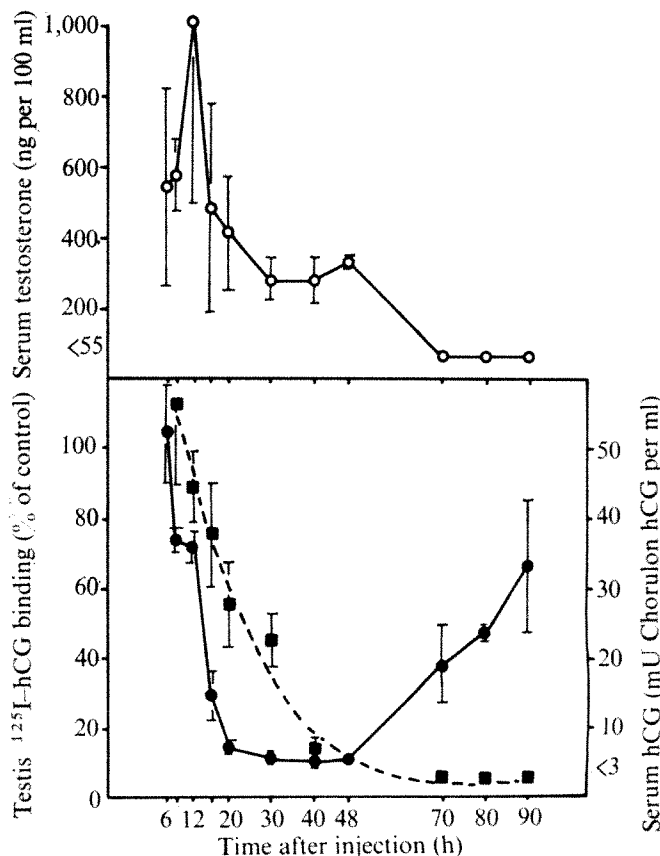


Fig. 1 *In vitro* binding of ¹²⁵I-hCG by testis homogenates in relation to serum levels of hCG and testosterone in hCG-treated immature rats. Twenty-one-day-old L/H rats were injected subcutaneously with either 10 IU hCG (Chorulon) in 0.5 ml 0.9% saline or with the vehicle. Pairs of saline-treated (control) and hCG-treated rats were killed at various intervals after injection. Animals were anaesthetised with ether, blood collected by decapitation and the paired testes removed and decapsulated in Krebs–Ringer bicarbonate solution (KRB). Testes from each individual rat were gently homogenised in 2 ml KRB in a glass homogeniser, filtered through a single thickness of gauze and the filtrate centrifuged at 1,500g for 5 min. The pellet was then weighed and resuspended in KRB at a concentration of 100 mg tissue ml⁻¹ and stored at –20 °C. When all samples had been collected, homogenates were thawed, resuspended and 0.2 ml added to 7 ml polystyrene tubes, which were then incubated in duplicate with ¹²⁵I-hCG (approximately 50,000 c.p.m. in 0.1 ml KRB containing 0.2% bovine serum albumin, fraction V) for 3 h at 37 °C. Non-specific binding of ¹²⁵I-hCG by each homogenate was determined by incubation of tubes containing 100 IU hCG. Incubation was terminated by addition of 5 ml cold 0.9% saline. Tubes were then centrifuged at 1,500g for 20 min, the supernatant decanted and radioactivity in the pellet measured in a gamma spectrometer. The ¹²⁵I-hCG was hCG CR119 (11,600 IU mg⁻¹) iodinated by the Chloramine-T method to a specific activity of approximately 40–50 μ Ci μ g⁻¹; the labelled hCG was purified on a column of cellulose CF11. Nonspecific binding was 0.5–1.6% of the total counts added. Specific binding, computed as the counts bound in the absence of unlabelled hCG minus the nonspecific binding, was 5–6% for control immature rats (20–27% for comparable adult tissue). ●, binding of ¹²⁵I-hCG in hCG-treated rats expressed as a % of the mean binding by tissue from the respective controls. Binding was reduced significantly ($P < 0.05$) at all times other than at 6 h, compared with controls. ○, Serum testosterone; ■, serum hCG. Vertical bars represent \pm 1 s.d.

Table 1 Effect of varying doses of injected hCG on the *in vitro* binding of ¹²⁵I-hCG 42 h later

hCG injected (IU)	¹²⁵ I-hCG bound c.p.m.	% of control	Serum testosterone (ng per 100 ml)
0	1,610 \pm 178	(100)	< 56
10	193 \pm 83	12.0 \ddagger	166 \pm 9
4	468 \pm 88	29.1 \ddagger	129 \pm 83
1	883 \pm 249	54.8*	124 \pm 38
0.1	1,044 \pm 144	64.8*	67 \pm 49
0.01	1,000 \pm 68	62.1 \ddagger	95 \pm 24

There were three animals per treatment group. Experimental procedures were as described in Fig. 1, except that only 10 mg fresh homogenate per tube was used for incubations.

Values are mean \pm 1 s.d. * $P < 0.02$; $\ddagger P < 0.01$; $\ddagger\ddagger P < 0.001$, compared with control.

8 h after injection of ^{125}I -hCG, and then remained fairly constant until 40 h (Fig. 2), suggesting that an equilibrium had been established. Injection of 10 IU hCG apparently disrupted this equilibrium, initially stimulating the uptake of ^{125}I -hCG and then lowering it (Fig. 2). Receptor occupancy, as judged by the actual level of radioactivity in the testes, increased between 4 and 8 h after injection but then fell by over 90% between 8 and 40 h in both treatment groups (data not shown). Labelled hCG in the blood showed a similar disappearance curve to that shown for hCG in Fig. 1.

Reduced binding of ^{125}I -hCG to testis tissue from hCG-treated rats could be due simply to saturation of the receptors by injected hCG; in this case one would expect an inverse relationship between binding of ^{125}I -hCG *in vitro* and the degree of receptor occupancy. This expectation does not match the findings, as receptor occupancy and the binding of ^{125}I -hCG *in vitro* decreased together between 8 and 40 h after injection of hCG. This suggests that hCG has caused a reduction in the actual number of hCG-receptors in the testis. Again, findings *in vivo* show a distinct difference in the ability of the testis to bind ^{125}I -hCG before and after exposure to hCG. In the first 8 h after injection, hCG stimulated the *in vivo* uptake of ^{125}I -hCG, probably by increasing testicular blood flow¹⁰; in contrast, at 40 h, when uptake had fallen well below the maximum, further injection of hCG/ ^{125}I -hCG failed to increase uptake of the labelled hormone 8 h later ($n=3$; saline-treated at 40 h, $T/B=0.81\pm0.21$; hCG/ ^{125}I -hCG-treated, $T/B=0.60\pm0.06$). This finding would seem to confirm that exposure to hCG results in impairment of testicular hCG binding by reducing the availability of receptors. Furthermore, as this effect is less pronounced following short term treatment with hCG, and is dose dependent, it seems likely that the extent of receptor loss is determined by the degree of exposure to hCG.

The observation that both short and long term exposure to hCG stimulated testosterone release to a similar extent may mean that the steroidogenic response of the testis is restricted to the first 12 h or so after injection; the gradual decline in testosterone levels between 12 and 70 h after injection of hCG might then reflect clearance of this steroid. Alternatively, the ability of the testis to secrete testosterone may have fallen as a result of the decrease in receptor availability, as animals given two injections of hCG 42 h apart show no significant elevation of testosterone 6 h after the second injection (unpublished data).

Similar results have been reported in the hypophysectomised female rat¹¹, where treatment with hCG or LH caused a reduction in hCG-binding by the ovary *in vitro* and *in vivo* up to 90 h later. Again, ovarian LH/hCG receptors in cultured follicles have been shown to become refractory to further ovine LH stimulation following an

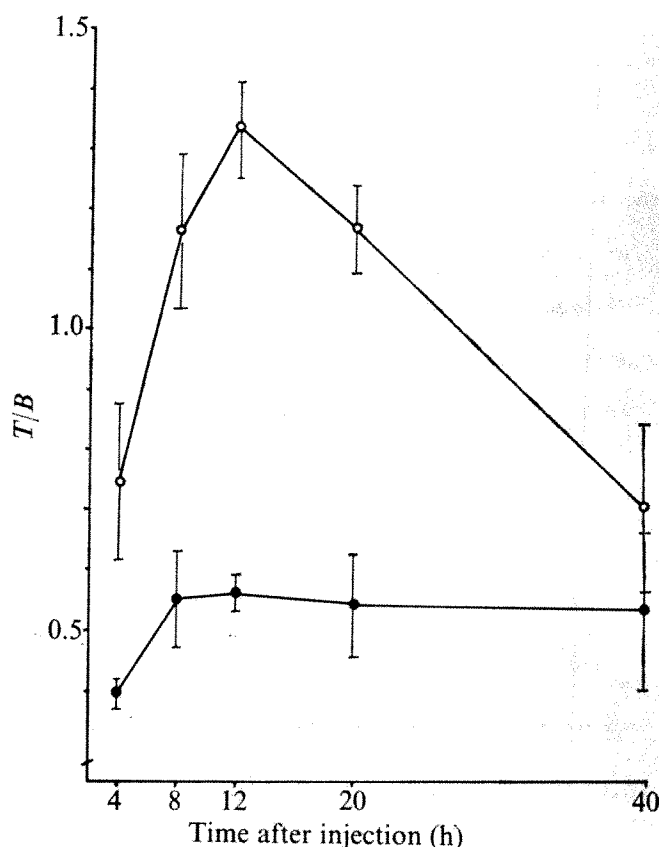


Fig. 2 Effect of injected hCG on the *in vivo* binding of ^{125}I -hCG by immature rat testes. Injections were given subcutaneously in 0.5 ml 0.9% saline. Animals were killed with ether at intervals after injection, 0.2 ml blood obtained by cardiac puncture and the paired testes removed and cleaned of extraneous fat. Blood and testes were placed in tared tubes, weighed, and radioactivity measured in a gamma spectrometer. The ^{125}I -hCG injected was prepared as described in Fig. 1, and retained full biological activity, as judged by the ability of equal amounts of labelled and unlabelled hCG to stimulate similar release of testosterone *in vitro* by decapsulated rat testes⁶. Preliminary experiments showed that, after injection of 0.5 μCi ^{125}I -hCG, the concentration of radioactivity in the testes was between 3.3 and 4.5 times higher than that in muscle at 2–40 h. Results are plotted as the ratio of radioactivity per mg testis over the radioactivity per mg blood. ●, Injected with ^{125}I -hCG only (three to four animals per point); ○, injected with ^{125}I -hCG together with 10 IU hCG (two to three animals per point). Vertical bars represent ± 1 s.d.

initial exposure to ovine LH¹², while LH has also been implicated in the loss of LH-receptors during luteinisation¹³.

These results suggest that binding of hCG to specific gonadotropin receptors in the rat testis results in changes in the subsequent availability of these receptors which, in

Table 2 Effect of injected antiserum to hLH on *in vitro* testis binding of ^{125}I -hCG in hCG-treated rats

Experiment	No. of animals	Treatments		Time when killed (h)	^{125}I -hCG bound		Serum testosterone (ng per 100 ml)
		1	2		c.p.m.	% of respective control	
a	2	Saline	—	20	3,249 \pm 115	—	< 35
	2	Saline	anti-hLH		3,104 \pm 65	—	< 50
	2	hCG	—		462 \pm 2	14.2 \ddagger	414 \pm 156
	4	hCG	anti-hLH		1,905 \pm 136	61.4 \ddagger	405 \pm 158
b	2	Saline	anti-hLH	30	2,932 \pm 42	—	< 43
	2	hCG	—		342 \pm 28	11.7 \ddagger	277 \pm 57
	2	hCG	anti-hLH		905 \pm 117	30.9 \ddagger	193 \pm 55

After the initial injection of saline or 10 IU hCG, second injections were given either 4 h (Exp. a) or 8 h (Exp. b) later. All injections were given subcutaneously in 0.5 ml 0.9% saline. Animals were killed at 20 h (Exp. a) or 30 h (Exp. b) after the first injection. Other details are as shown for Fig. 1 except that testis homogenates were used fresh for uptake studies. A 1 : 10 dilution of serum from saline/antiserum-treated rats bound 66.3 \pm 4.9% (20 h) and 56.9 \pm 0.4% (30 h) of added ^{125}I -hLH under radioimmunoassay conditions; serum (1 : 10) from hCG/antiserum-treated rats bound 56.1 \pm 1.0% (20 h) and 51.8 \pm 1.0% (30 h) of added ^{125}I -hLH. Figures are the mean \pm 1 s.d.

$\ddagger P < 0.01$, $\ddagger\ddagger P < 0.001$ compared with respective control.

turn, may limit the steroidogenic potential of the testis, and may represent a way in which a target tissue can regulate its sensitivity to hormonal stimulation¹.

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Interactions of cholesterol with the Na pump in red blood cells

It is well established that cholesterol, by interacting with phospholipids, decreases the permeability and increases the physical resistance of natural and artificial membranes^{1–3}. In contrast, studies of the interactions of cholesterol with membrane proteins have yielded variable results, depending on the experimental conditions and/or the enzymatic activity tested. For example, in cholesterol-enriched mitochondria, an enhancement of activity is seen only in the case of succinate-cytochrome *c* reductase⁴. The activity of certain isolated membrane-bound enzymes involved in ionic transport is affected by the presence of cholesterol. Between 50 and 90% inhibition of the phospholipid-induced $(\text{Na}^+ + \text{K}^+) - \text{Mg}^{2+} - \text{ATPase}$ activation^{5,6} and complete inhibition of the $(\text{Ca}^{2+}) - \text{Mg}^{2+} - \text{ATPase}$ activity⁷ have been reported when cholesterol is added to the phospholipid environment of isolated enzymes. This observation suggested that cholesterol is excluded from the membrane areas containing the transport enzymes. Data concerning the Na pump are also conflicting. In cholesterol-depleted red blood cells, some workers found that the pump-mediated K influx is decreased⁸ whereas, according to others, the pump-mediated Na efflux is increased⁹. Because in physiological conditions these fluxes should be stoichiometrically coupled^{10,11}, the observations are contradictory. In addition, it has been reported that the pump-mediated Na efflux in cholesterol-enriched red cells is reduced for guinea-pig cells¹² but unchanged for human cells⁹. We have investigated the effects of cholesterol depletion on the kinetic properties of the well characterised Na pump of human

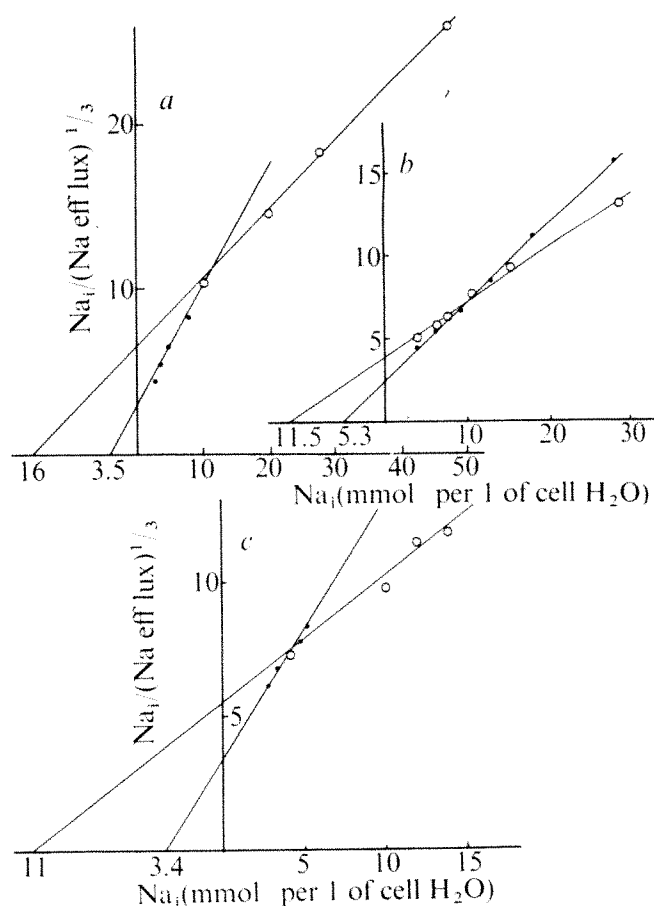


Fig. 1 Effect of internal Na concentration on the ouabain-sensitive Na efflux from control RBC (●) and from cholesterol-depleted RBC (○) plotted according to equation (2). *a* and *b*, Cells were suspended in 10 mM K media (exchange Na–K); *c*, cells were suspended in K-free media (exchange Na–Na). Changes in Na_i were achieved either by incubation at 37 °C in media of different Na concentration (25, 80, 100 and 140 mM) (*a* and *c*), or by treatment with PCMBs and media of various Na concentrations (0, 5, 10, 20, 30 and 50 mM). One typical experiment is shown in each case.

red blood cells (RBC). The turnover of the Na pump and the apparent affinities for internal and external cations were calculated according to a kinetic model described before¹³. Our results show that, depending on the experimental conditions, cholesterol may behave either as an activator or as an inhibitor, thus explaining previous contradictory observations. Moreover, our findings suggest that, in intact membranes, cholesterol is not excluded from the microenvironment of the Na pump.

RBC recovered from freshly drawn human blood were depleted of cholesterol by 15 h of incubation (37 °C, haematocrit 10%) in lecithin vesicle suspensions (L vesicles, 20 mg of lecithin per ml of cells) according to the technique of Bruckdorfer *et al.*¹⁴. Controls were incubated in the same conditions either in mixed cholesterol–lecithin vesicle suspensions (CL vesicles, molar ratio

Table 1 Changes of the RBC membrane lipid content during incubation with lipid vesicle suspensions

	Cholesterol (mg per g of haemoglobin)	Phospholipids (mg per g of haemoglobin)	Cholesterol Phospholipids (mol/mol)	Cholesterol depletion (%)
Nonincubated cells	3.62 ± 0.20	9.20 ± 0.02	0.77	
Incubated cells				
Controls (CL vesicles)	3.70 ± 0.40	9.16 ± 0.08	0.79	0
Cholesterol-depleted (L vesicles)	2.34 ± 0.28	9.07 ± 0.10	0.51	36 ± 9

Table 2 Effects of cholesterol depletion on the maximal rate of Na efflux (M_{\max}) and on the apparent dissociation constant for internal Na (K_{Na}')

	PCMBS	M_{\max} (mmol l ⁻¹ h ⁻¹)	10 mM external K K_{Na}' (mM)	Cholesterol depletion (%)	M_{\max} (mmol l ⁻¹ h ⁻¹)	K-free medium K_{Na}' (mM)	Cholesterol depletion (%)
Controls	—	2.1	3.5	0	1.2	3.4	0
	+	9.7	5.3	10			
Cholesterol depleted	—	14.2	16.0	41	7.1	11.0	28
	+	26.8	11.5	—*			

M_{\max} and K_{Na}' were calculated according to equation (2) from the intercept and slope of the lines of Fig. 1.

* Not measured.

0.85, 4 mg lipids per ml of cells), or in vesicle-free media. Vesicle suspensions were prepared by sonication of either egg lecithin (L vesicles) or cholesterol plus egg lecithin (CL vesicles) for 1 h at 4 °C under nitrogen, and centrifuged for 1 h at 100,000g. Adenine (2 mM) and inosine (10 mM) were added during the last hour of incubation to replenish the energy stores of the cells. The composition of the medium was (mM): (NaCl + KCl), 150; MgCl₂, 1; Na₂HPO₄–NaH₂PO₄, 2.5 (pH 7.4); glucose, 10; penicillin, 5,000 UI ml⁻¹. Modifications of the internal Na (Na_i) were induced by using media with different Na concentrations during the 15 h of incubation. Alternatively, after the 15 h of treatment at constant external Na, another 12-h incubation was carried out in media with 0.1 mM *para*-chloromercuribenzenesulphonate (PCMBS) and variable Na concentrations¹⁵. Na and K intracellular concentrations and fluxes were measured by a procedure similar to that described by Garrahan and Glynn^{10,11}. Ouabain-sensitive Na efflux was measured as a function of Na_i in control and cholesterol-depleted RBC, either in the presence of 10 mM external K (where the Na efflux is coupled to a K influx: Na–K exchange) or in K-free media (where Na efflux is coupled to a Na influx: Na–Na exchange). As previously reported¹³, the activation curve of Na efflux from red cells can be described adequately by a simple kinetic model. This model assumes that Na translocation will take place only in pump units which have three equivalent and independent sites occupied by Na_i . Therefore the Na efflux (M) obeys the following equation

$$M = \frac{M_{\max}}{(1 + K_{Na}'/Na_i)^3} \quad (1)$$

where M_{\max} is the Na efflux at saturating Na_i and K_{Na}' the apparent dissociation constant of the Na_i -site complex. Equation (1) can be transformed into

$$\frac{Na_i}{M^{1/3}} = \frac{K_{Na}'}{M_{\max}^{1/3}} + \frac{Na_i}{M_{\max}^{1/3}} \quad (2)$$

RBC lipids were extracted in chloroform–isopropanol¹⁶. Cholesterol was measured after Liebermann–Burchard coloured reactions and total lipid phosphorus was determined after removal of water-soluble phosphorus¹⁷.

No significant change was observed in the RBC cholesterol content after incubation in CL vesicle suspensions (Table 1) or in vesicle-free media (results not shown). In contrast, when incubated in L-vesicle suspensions, the cells lost about 36% of their cholesterol (Table 1). In all conditions, the total phospholipid content remained unchanged, so that the molar cholesterol : phospholipid ratio decreased from 0.8 in control to 0.5 in depleted cells (Table 1).

Plots of the function $Na_i/M^{1/3} = f(Na_i)$ (equation (2)) are shown in Fig. 1a and b (exchange Na–K, 10 mM external K) and in Fig. 1c (exchange Na–Na, K-free medium). In Fig. 1a, the range of Na_i obtained without PCMBS, was considerably larger in depleted RBC than in the controls. This agrees with previous evidence that cholesterol removal induced an increase

in the passive permeability of the membrane¹. In contrast, in Fig. 1b, comparable Na_i values were obtained in both types of cell after treatment with PCMBS. In all cases, straight lines are observed, confirming the three-site kinetic model for internal Na, even in situations far from the physiological state, as in cholesterol-depleted membranes. The maximal rate of Na efflux (M_{\max}) and the apparent dissociation constant for internal Na (K_{Na}') were calculated from slopes and intercepts of the lines of Fig. 1a, b and c in each experimental condition. In both cases (Na–K and Na–Na exchanges), cholesterol depletion increases the maximal rate of Na efflux (six to seven times) and the apparent dissociation constant for internal Na (three to five times) (Table 2). The kinetic parameters obtained in control cells treated with PCMBS do not coincide exactly with those of cells not so treated (Table 2). This can probably be explained by an additional effect of PCMBS—still to be evaluated—such as a slight decrease in the cholesterol content, as shown in Table 2. Therefore in our experimental conditions cholesterol behaves as an inhibitor of pump turnover and an activator of the apparent affinity for internal Na. The points where the lines cross each other (6–12 mM) define the internal Na concentrations at which cholesterol depletion has no apparent effect on the ouabain-sensitive Na efflux. Below these Na_i values, cholesterol depletion decreases Na efflux whereas above them, it increases Na efflux. It thus seems that an equivalent cholesterol depletion may induce either a decrease or an increase in pump activity. The opposite effects observed by different experimenters would result from differences in the internal Na content.

Since cholesterol alters the apparent affinity of the pump for internal Na, it was interesting to test whether it also affects that for external cations. Apparent affinities were determined by varying external K or external Na at constant internal Na and K. No change in apparent affinities was detected in cholesterol-depleted cells compared with control cells in conditions of either Na–K exchange or Na–Na exchange. This result indicates that the cholesterol effect is specific for the internal sites of the Na pump.

The enhancement of the maximal rate of the Na pump induced by cholesterol depletion may be due to an overall decrease of the internal viscosity of the lipid core of the membrane, as the following observations suggest: (1) addition of cholesterol either in aqueous dispersions of phospholipids or into RBC membranes increases the internal viscosity of the membrane^{18,19}; (2) isolated purified transport enzymes such as (Ca²⁺)–Mg²⁺–ATPase or (Na⁺ + K⁺)–Mg²⁺–ATPase seem to require a fluid phospholipid environment for optimal activity^{5,7}. This interpretation does not imply that, in depleted cells, cholesterol is removed from the immediate environment of the pump, for a fluidising effect could occur even at a distance. In contrast, a change in the apparent affinity of the pump, as we have detected for internal Na, and which is linked to a conformational change of the protein or to another physicochemical property responsible for the binding, could suggest a direct interaction of cholesterol with the pump. Taking into account the previous experimental evidence for the simultaneous existence of external and internal cation sites on the same pump unit²⁰ and for the quaternary structure of the (Na⁺ + K⁺)–Mg²⁺–ATPase^{21,23}, the specific effect of cholesterol can be interpreted, assuming that

the external and internal cation sites are in different subunits, and that cholesterol interacts only with the internal subunit.

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Anti-actin stains synapses

SMOOTH muscle antibody (SMA) found in the sera of some patients with active chronic hepatitis (ACH)¹ has been shown to contain specific anti-actin antibody^{2,3}. These SMA sera have been used to demonstrate the presence of the corresponding antigen in normal² and neoplastic^{4–6} 'non-muscle' tissues. We have demonstrated that SMA serum reacts with normal, neoplastic and foetal astrocytes but not with neurone cell bodies^{5,7}. The present report deals with the reaction of SMA with synaptic endings in the central nervous system (CNS).

The neural tissues examined comprised cerebellum and cerebrum from rat, mouse, rabbit, guinea pig, sheep and

chicken, and spinal cord from rat, mouse and chicken. Synaptosome⁸- and myelin⁹-enriched fractions were also isolated from chicken forebrain; effective enrichment was established by electron microscopy and by enzyme marker assays^{8,9}. The fractions were washed three times in phosphate-buffered saline (0.145 M NaCl, 0.01 M sodium phosphate, pH 7.1), resuspended in a minimum volume of the same medium, smeared on glass slides and air-dried at 4 °C for 2 h. The sections and smears were tested with SMA by standard sandwich immunofluorescence (IFL) tests¹⁰.

The SMA serum⁴ from a patient with ACH was characterised by reactivity with the following tissues: smooth muscle¹, skeletal muscle striations^{2,3}, liver in a 'polygonal' pattern², renal glomeruli in a diffuse pattern², the brush borders and peritubular fibrils of renal tubules^{2,6} and thymus medulla². The conjugate used to trace any bound immunoglobulin was a fluorescein-isothiocyanate (FITC)-labelled goat anti-human gammaglobulin with a fluorescein-to-protein molar ratio of 4.0 and a protein content of 0.8 g per 100 ml. Before use, it was appropriately absorbed⁵ so that by itself it gave no staining reaction on test sections or smears. Specificity tests were performed by reacting sections and smears with SMA serum absorbed¹⁰ with pellets of synaptosomal or myelin fractions or absorbed with skeletal muscle actin, myosin, tropomyosin or troponin¹¹. Purity of the isolated muscle proteins was established by polyacrylamide gel electrophoresis¹¹. The precipitate formed by adding SMA to actin was eluted with 0.1 M glycine-HCl, pH 2.8 and the neutralised eluate used in IFL tests³.

In sections of mammalian and chicken cerebellum, the outer molecular layer showed bright staining of fine particles, about 2 µm in diameter (Fig. 1a and b), and the granular layer showed staining of similar particles clustered around ovoid bodies 10–20 µm in diameter (Fig. 1a and c). The white matter (Fig. 1a), the centres of some ovoid bodies (Fig. 1c), and Purkinje cell bodies (Fig. 1a) were negative. Astrocyte staining⁵ was seen in all three layers of the cerebellum but was best visualised between ovoid bodies in the granular layer (Fig. 1a) and in the white matter where its fluorescence was not masked by staining of the bright fluorescent particles. Cerebellar blood vessels were brightly fluorescent. To locate the site of the ovoid bodies in the granular layer precisely, a double immunofluorescence staining technique with rhodamine and FITC was used¹⁰; nuclei were stained with anti-nuclear factor and contractile protein with SMA. This experiment showed that the ovoid bodies were situated between the cell bodies of granule neurones and that the latter did not stain with SMA.

Cerebrum and spinal cord sections showed staining of fine particles in the grey matter indistinguishable from those seen in the cerebellum; the white matter and neurone cell bodies did not stain. Smears of synaptosomal fractions also

Table 1 Immunofluorescence staining of neural and non-neural tissues by SMA

	Staining titre against unfixed frozen sections and/or smears of chicken neural tissues*:					Staining titre against unfixed frozen sections of non-neural tissues:					
	CNS synaptic endings	Synaptosomes	Neurone cell bodies	Astrocytes	Myelin	Smooth muscle in mouse stomach	Rabbit skeletal muscle	Rat liver	Rat renal glomeruli	Rat renal tubules	Rat thymus
SMA											
Absorptions:											
Nil	64	64	< 8	64	< 8	256	64	128	256	64	64
Synaptosomes	< 8	< 8	< 8	< 8	< 8	16	< 8	< 8	16	< 8	< 8
Actin†	< 8	< 8	< 8	< 8	< 8	< 8	< 8	< 8	< 8	< 8	< 8
Myelin	64	64	< 8	64	< 8	256	64	128	256	64	64
Myosin‡	64	64	< 8	64	< 8	256	64	128	256	64	64

* Results with sections of mammalian neural tissues were indistinguishable from those obtained with the chicken.

† Immunoabsorption was carried out by overnight incubation at 4 °C of 0.1 ml SMA serum with 4.0 mg skeletal muscle protein.

‡ Immunoabsorption of SMA serum with 4 mg tropomyosin or troponin also did not reduce the staining titres for neural or non-neural tissues.

showed staining of small (about $2\ \mu\text{m}$) particles (Fig. 1d) while myelin smears were negative.

Immunoabsorption studies (Table 1) showed that SMA staining of neural and non-neural tissues was inhibited by serum absorption with synaptosomal but not myelin fractions and by skeletal muscle actin but not by myosin, tropomyosin or troponin. The neutralised eluate obtained by acid dissociation of the SMA-actin precipitate gave the same staining pattern in neural and non-neural tissues as the original serum.

We conclude that the discrete particles in neural tissues stained by SMA are synaptic endings, based on the following observations: (1) the particle size (about $2\ \mu\text{m}$) in CNS sections *in vivo* and in smears of synaptosomal fractions *in vitro* corresponds to the average diameter of synaptic endings; (2) they are located in the grey matter where synapses are abundant, and not in white matter and (3) particle staining is inhibited by serum absorption with synaptosomal and not myelin fractions. Further, the size of these particles in the CNS is similar to that revealed by staining with antibodies raised in rabbits against chicken synaptic plasma membrane⁸ or synaptic vesicle¹² fractions. The size (about $10\text{--}20\ \mu\text{m}$) and location of the particle-studded ovoid bodies in the cerebellar granular layer, and the inhibition of their staining by serum absorption with synaptosomal and not myelin fractions suggest that these are 'cerebellar glomeruli'—ovoid-shaped structures of about

$20\ \mu\text{m}$ in their greatest dimension, situated between granule neurones, and made up of synaptic complexes of central mossy fibre axons and the dendrites of granule and Golgi neurones¹³. The absence of SMA reactivity with CNS white matter indicates that antigen is not present in neuronal axons or myelin sheaths; the negative staining seen in the centre of ovoid bodies may correspond to the mossy fibre axon around which synapses are formed. The present results show that, in neurones, contractile protein antigen is restricted to synaptic endings and absent from cell bodies and axons; these observations support the results of one ultrastructural study of actin in the CNS¹⁴ but are at variance with those of another¹⁵.

Immunoabsorption studies of SMA serum with subcellular brain fractions and muscle proteins indicate that an actin-like protein is the antigen demonstrated in synaptic endings; this was confirmed by obtaining the same staining pattern with the eluate obtained by acid dissociation of the SMA-actin precipitate. The present results provide immunohistological confirmation for the presence of actin-like protein in synaptic endings—demonstrated previously by actin isolation from synaptosomal fractions of bovine and rat brains¹⁶. In our experience, SMA provides a more precise means of localising contractile protein in synaptic endings than can be obtained with antisera raised in rabbits against brain actomyosin-like proteins¹⁷.

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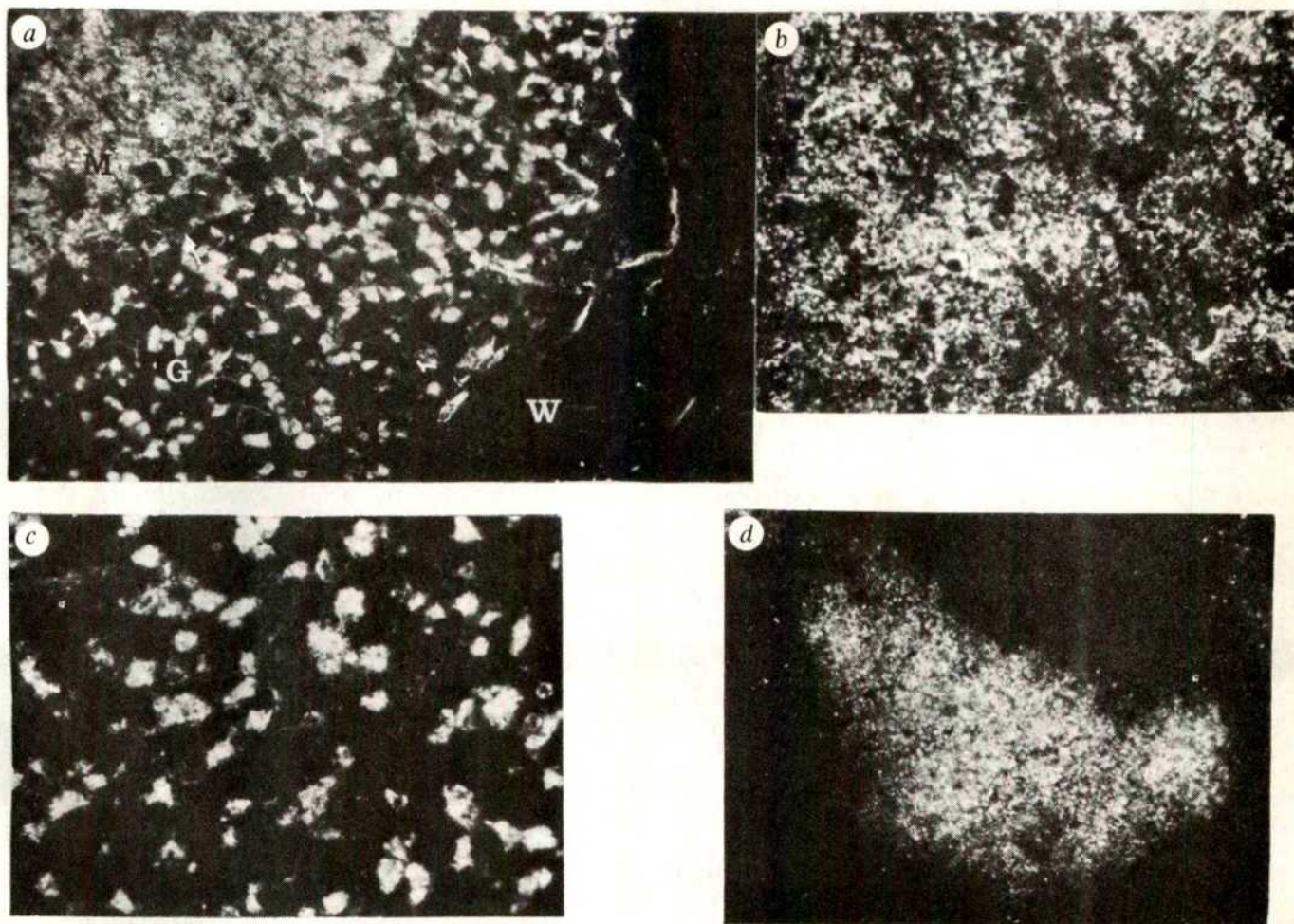


Fig. 1 Indirect immunofluorescence staining of neural tissues by human smooth muscle autoantibody. *a*, Rat cerebellum cryostat section showing bright staining of fine particles ($2\ \mu\text{m}$) in the outer molecular layer (M) and of ovoid bodies ($10\text{--}20\ \mu\text{m}$) in the granular layer (G). The white matter (W) and neurone cell bodies are not stained; the negative cell bodies of Purkinje cells are arrowed. The stained cell body and processes of granular layer astrocytes are also seen in this field ($\times 272$). *b*, Chicken cerebellum cryostat section showing fine particulate staining of the outer molecular layer; neurone cell bodies are not stained ($\times 425$). *c*, Chicken cerebellum cryostat section showing fine particles clustered around ovoid bodies of the granular layer; the centres of some ovoid bodies are not stained ($\times 425$). *d*, Chicken synaptosome smear showing fine particulate staining ($\times 425$).

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Hypersensitivity to purified brain proteins in healthy individuals

DURING the course of our research in multiple sclerosis it was observed that apparently healthy laboratory personnel who had been artificially and accidentally exposed to brain tissue showed patterns of hypersensitivity inconsistent with our basic control group. We determined at that time to study them further as a separate group. Twelve people have been identified and studied. We subsequently categorised the types of exposure into three classes as shown in Table 1.

We show here that these individuals develop cellular sensitivity to the brain antigens to which they were exposed, and that the sensitivity is actively blocked in autologous serum by a humoral factor.

The method of lymphoblastic transformation as measured by tritiated thymidine uptake was used throughout. Lymphocytes were isolated from defibrinated peripheral blood by the Ficoll-Hypaque (LSM-Bionetics) method and cultured in RPMI-1640 medium with Hepes buffer, 1% glutamine and penicillin-streptomycin or gentamicin. The antigens used were myelin basic protein (BP) and glial fibrillary acidic (GFA) protein^{1,2} along with control antigens *Candida albicans* (Hollister-Stiers) and purified tetanus antigen. Microtitre plates (Cooke Engineering) were utilised for cultures (400,000 cells in 0.2 ml per well), which were incubated for 6, 7 or 8 d at 37 °C under humid 5% carbon dioxide environment. These cultures were set up in homologous (sex and blood-type matched pools), as well as autologous sera at 10% concentration. The cultures were collected with the Multiple Automated Sample Harvester

(MASH-II, Microbiological Associates) 18-20 h after the addition of 1 μ Ci per well of tritiated thymidine (New England Nuclear). After drying 2 h at 80 °C, the filter paper disks were counted in 5 ml of Liquifluor scintillation cocktail (New England Nuclear) in a Packard liquid scintillation counter. The average counts per minute (c.p.m.) of triplicate determinations were expressed as stimulation index, that is the ratio of average c.p.m. of antigen-exposed cultures to average c.p.m. of antigen-free cultures.

Table 1 Types of exposure		
Type	Number	Description
Purification	6	Individuals who were exposed while purifying brain proteins
Autopsy	5	Individuals who were exposed while performing or assisting with autopsies
Injection	1	Individual who accidentally injected self intracutaneously with whole guinea pig myelin homogenised in complete (H37Ra added) Freund's adjuvant

Recognising that individual variations do exist in the temporal response to brain protein (that is, 6, 7, or 8 d of culture), we are presenting only those data obtained on the day of maximum response for each concentration of antigen. The maximum stimulation index for each antigen and concentration was compared with the maximum stimulation index of corresponding antigen and concentration of 13 control individuals who had no determinable history of exogenous exposure to brain or brain components. No significant differences in antigen-free cultures were noted between cells cultured in autologous sera and homologous sera. The data are shown in Table 2.

The most striking observation in the Purification group is the apparent lack of response of these people who have had constant and intense exposure to brains and their

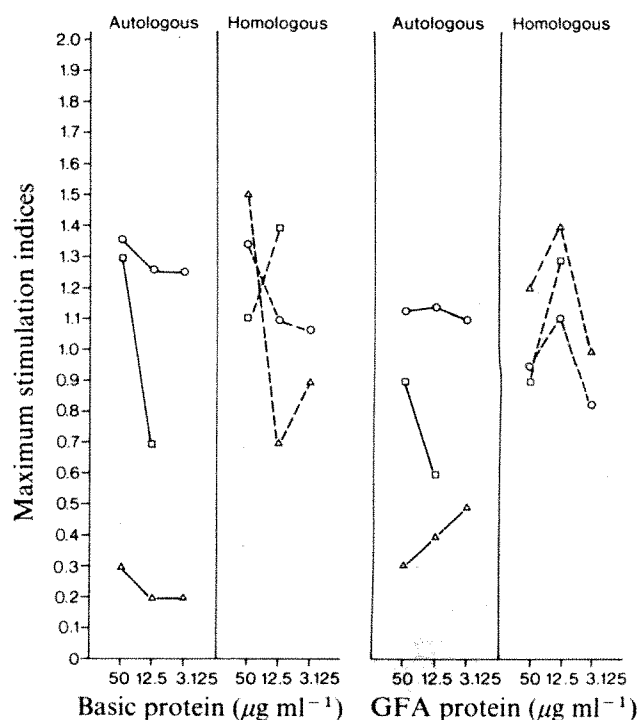


Fig. 1 Stimulation indices of cells from accidentally injected individual at 10 and 38 d post-injection, against myelin basic protein and glial fibrillary acidic protein in autologous and homologous sera. \circ , Average stimulation indices of controls; \triangle , average stimulation indices at 10 d post-injection; and \square , average stimulation indices at 38 d post-injection. — Autologous sera; --- homologous sera.

isolated component proteins (BP and GFA) when tested in their own sera. The underlying cellular response is unmasked in homologous sera and is significantly increased (dose maxima noted at BP 50 $\mu\text{g ml}^{-1}$ and GFA 12.5 $\mu\text{g ml}^{-1}$). Of great interest is the relative depression in autologous sera (reaching only slight significance, $P < 0.07$), at GFA 50 and titrating back to insignificance at GFA 3.1 ($P < 0.5$). We interpret this to be evidence for active blocking factors in autologous sera. It should be noted that all the subjects in this group are from a single laboratory concerned primarily with the isolation and characterisation of GFA protein.

The Autopsy group, on the other hand, have been exposed primarily to whole brain tissue. Here the basic cellular response (in homologous sera) is clearly significant against BP, and becomes apparent even in autologous sera, suggesting that the blocking factor found in the Purification group may be either decreased or less reactive in the Autopsy group. The response to GFA shows the typical pattern of significantly enhanced response, detectable only in the homologous sera (a slightly enhanced reaction to GFA 12.5 $\mu\text{g ml}^{-1}$ in autologous sera is present, but does not reach significance; $P < 0.09$).

By far, the most intriguing case is the one individual who accidentally injected herself in the finger (6/8/76) with whole guinea pig myelin homogenised in complete (H37Ra added) Freund's adjuvant. At 5 d post-injection she had massive swelling of the finger and hand, but to date has not developed any signs of neurological damage. Injections

Table 3 Average maximum stimulation indices of injection exposure against BP and GFA proteins

Type Injection	Serum	BPC*	Stimulation index† (at 10 and 38 d post-injection)	
			10	38
Auto	Homo	BP 50.0	0.3	1.3
		BP 12.5	0.2	0.7
		BP 3.1	0.2	—
		BP 50.0	1.5	1.1
		BP 12.5	0.7	1.4
		BP 3.1	0.9	—
	Auto	G 50.0	0.3	0.9
		G 12.5	0.4	0.6
		G 3.1	0.5	—
		G 50.0	1.2	0.9
		G 12.5	1.4	1.3
		G 3.1	1.0	—

*Brain protein and concentration; BP is Kies myelin basic protein, G is glial fibrillary acidic protein. Concentration is expressed in $\mu\text{g ml}^{-1}$.

†Maximum stimulation index (see text).

of this sort are known to cause experimental allergic encephalomyelitis (EAE) in monkeys³ and most other mammals⁴ within 2–3 weeks after injection. We first tested her at 10 d (the swelling had subsided almost completely at this time) and subsequently exactly one month later (38 d post-injection) (Table 3 and Fig. 1.) Her initial response to the injected tissue seems to be one of severe depression in autologous serum (note that the cells have normal re-

Table 2 Average maximum stimulation indices of purification and autopsy exposure groups against BP and GFA proteins

Type	Serum	AMRD*	BPC†	AMSI‡	SD	N	Significance§	
Control	Auto	6.8	BP 50.0	1.36	±0.63	10		
			BP 12.5	1.25	0.28	13		
			BP 3.1	1.26	0.51	10		
	Homo	7.0	BP 50.0	1.34	0.42	11		
			BP 12.5	1.11	0.30	12		
			BP 3.1	1.09	0.45	8		
	Auto	6.9	G 50.0	1.13	0.35	10		
			G 12.5	1.14	0.46	10		
			G 3.1	1.11	0.33	7		
	Homo	7.0	G 50.0	0.95	0.33	12		
			G 12.5	1.10	0.37	12		
			G 3.1	0.83	0.28	9		
Purification	Auto	6.7	BP 50.0	1.73	1.03	6	Not significant	$P < 0.4$
			BP 12.5	1.52	0.86	5	Not significant	$P < 0.35$
			BP 3.1	1.18	0.21	4	Not significant	$P > 0.5$
	Homo	6.6	BP 50.0	2.92	1.43	6	Very highly significant	$P < 0.01$
			BP 12.5	1.56	0.74	5	Slightly significant	$P < 0.08$
			BP 3.1	1.90	1.45	4	Not significant	$P < 0.17$
	Auto	7.1	G 50.0	0.70¶	0.49	6	Slightly significant¶	$P < 0.07$
			G 12.5	0.78¶	0.31	4	Not significant¶	$P < 0.18$
			G 3.1	1.05¶	0.59	4	Not significant¶	$P > 0.5$
	Homo	6.6	G 50.0	1.67	1.23	6	Slightly significant	$P < 0.08$
			G 12.5	1.80	0.61	4	Significant	$P < 0.02$
			G 3.1	1.55	1.41	4	Not significant	$P < 0.16$
Autopsy	Auto	6.5	BP 50.0	2.58	1.06	5	Significant	$P < 0.02$
			BP 12.5	1.80	0.88	5	Slightly significant	$P < 0.06$
			BP 3.1	1.60	1.27	2	Not significant	$P > 0.5$
	Homo	6.6	BP 50.0	4.25	4.85	4	Slightly significant	$P < 0.06$
			BP 12.5	2.15	1.46	4	Significant	$P < 0.04$
			BP 3.1	0.80	0.00	2	Not significant	$P > 0.5$
	Auto	6.8	G 50.0	1.60	1.06	5	Not significant	$P < 0.22$
			G 12.5	1.94	1.24	5	Slightly significant	$P < 0.09$
			G 3.1	0.95	0.92	2	Not significant	$P > 0.5$
	Homo	6.7	G 50.0	1.55	0.41	4	Significant	$P < 0.02$
			G 12.5	1.20	0.35	4	Not significant	$P > 0.5$
			G 3.1	2.4	—	1	Unknown	

*Average maximum response day (all concentrations of protein).

†Brain protein and concentration; BP is Kies myelin basic protein, G is glial fibrillary acidic protein. Concentration is expressed in $\mu\text{g ml}^{-1}$.

‡Average maximum stimulation indices (see text).

§Significance determined by Student's *t* test for two distributions.

¶Note that these are depressions rather than elevations.

activity in homologous serum for both tests) to both BP and GFA proteins. This is followed (at 38 d post-injection) by a tendency toward a "normal" response to BP and a somewhat persistent depression in the presence of GFA protein. Since this individual has worked for 15 yr with myelin basic protein and spinal cord tissue, these reactions probably represent a delayed hypersensitivity reaction in a previously sensitised individual.

We do not know the clinical significance of these results. All of these individuals are healthy and have no apparent symptoms of neurological or other physical problems. We propose that a delicate balance between cellular hypersensitivity and a humoral substance exists in these individuals and that a similar relationship is present in multiple sclerosis patients. The obvious question with respect to multiple sclerosis is: what is the basic difference between these individuals and those who display autoimmune disease? Colby *et al.*⁵ used lymphoblastic transformation against $10 \mu\text{g ml}^{-1}$ of BP at 20% homologous human plasma and reported a temporal relationship of enhanced reactivity to exacerbation. They detected the greatest reactivity within 3 weeks of the onset of exacerbation, while the least was found in patients more than 5 months after an acute episode. Sheremata *et al.*⁶, using macrophage inhibition assay, have evidence that sensitivity can indeed be detected before exacerbation. Our data suggest that an individual without multiple sclerosis will produce blocking factors that suppress the cellular response to exposure (and subsequent damage?) to nervous tissue. It remains to be determined if multiple sclerosis patients are either incapable of producing these blocking factors or produce ineffective ones. It is unclear whether the blocking factors demonstrated in our assay are identical to those observed by Van den Noort and Stjernholm^{7,8}.

At present, we are in the process of increasing our sample size to sort out any possible variables at work in this system: length of time of exposure, severity of exposure (whole brain only as against brain and its proteins), and sustained memory without subsequent exposure.

In conclusion, we would like to emphasise that when control groups are selected in studies involving brain proteins, one must be certain that their backgrounds do not include previous exposure to whole brain or its components, for this would certainly obscure and invalidate the results. Furthermore, when investigating cellular reactivity against different specific antigens, it is highly important to include various concentrations of the specific antigen, to vary the incubation period of the cell cultures and to set up such cultures in both autologous and homologous sera.

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Functional assessment of GABA uptake or exchange by synaptosomal fractions

THE discovery of a transport system in nervous tissue for an amino acid with an apparent K_m equal to or less than 10^{-5} M has been taken as support of a neurotransmitter function for this compound. "High affinity" systems of this sort have been described for γ -amino butyric acid (GABA)¹, glutamate², glycine³, aspartate⁴ and taurine⁵. Initially it was hoped that such transmitter transport systems could be used to map the utilisation of a given transmitter in defined anatomical regions. Unfortunately nonspecific elements, especially glial cells, also have the capacity to transport neurotransmitter candidates using "high affinity" systems⁶. Furthermore, Levi and Raiteri⁷, noting that "high affinity" transport was found where the internal concentration of the amino acid was high, suggested that isotopic measurements of inward transport were misleading and that exchange of labelled amino acid for unlabelled amino acid is likely to be the phenomena that has been measured⁸. This called into question the functional role of "high affinity" transport systems, and has resulted in considerable controversy⁹. In an effort to clarify the conditions of amino acid uptake, we have measured net GABA movement in nerve-terminal-enriched fractions¹⁰.

The conditions were chosen to maximise changes in the extracellular GABA concentrations and mimic as closely as possible the normal *in vivo* ratio of extracellular fluid to cells. Synaptosomes from one rabbit cortex were divided into six equal portions and each was incubated in a minimal volume (200 μl) of Tris-buffered glucose-saline media containing concentrations of GABA in the range 10^{-3} – 10^{-6} M. Experiments were carried out in media containing normal concentrations of sodium and potassium (140 mM Na⁺ and 3 mM K⁺) and in media containing increased K⁺ concentrations (91 mM Na⁺ and 52 mM K⁺), which would decrease both the membrane potential and ionic gradients. The synaptosomes were incubated for 20 min at 37 °C, which was sufficient to achieve equilibria in these conditions. They were sedimented by centrifugation and intra- and extracellular GABA concentrations were measured using GABA transferase and succinic semialdehyde dehydrogenase to generate NADH, which was measured spectrofluorometrically¹¹. The intracellular volume referred to is the portion of the pellet which was not penetrated by ¹⁴C-inulin. The role of GABA metabolism as a driving force was assessed by measuring the

Table 1 Changes in the external [GABA] on incubation of synaptosomes in various concentrations of GABA for 20 min

(GABA) Before Incubation in media (M)	(GABA) After 20 min Incubation	
	in media 10^{-3} M	in synaptosomes 10^{-5} M
10^{-3}	7.0 ± 0.3	111 ± 34
10^{-4}	5.6 ± 0.7	51 ± 17
10^{-5}	2.5 ± 0.4	41 ± 10
10^{-6}	2.1 ± 0.8	19 ± 7

Approximately 100 mg of synaptosomes was incubated in 200 μl of Tris-buffered glucose-saline medium for 20 min. Figures represent initial concentration, the concentration of GABA measured in the media after incubation and the GABA found in the synaptosomes. The synaptosomal content of GABA before incubation was $4.3 \pm 97 \times 10^{-4}$. The total amount of GABA present throughout the experiment was constant. Values are mean \pm s.d. from between three and seven experiments.

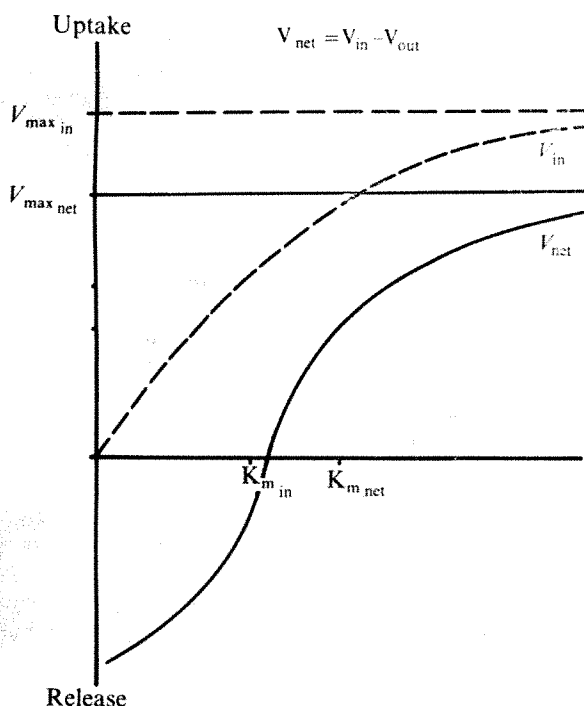


Fig. 1 Uptake or release velocity plotted against substrate concentration for a reversible transport system. This is compared with uptake using isotopic tracers which effectively measure inward flux (v_{in}). This demonstrates that apparent affinity of the substrate and the maximal rate will differ in experiments using isotopic tracer from experiments measuring the actual net rate.

total GABA in the system at the start of incubation and after equilibrium had been reached. The results in Table 1 indicate that GABA degradation is not appreciable and, therefore does not serve as a principal driving force in the system.

These results also indicate that synaptosomes incubated in our experimental conditions release GABA to the media at external GABA concentration of 10^{-6} and 10^{-5} M. At higher external GABA concentrations synaptosomes exhibit concentrative uptake. Thus, in the concentration range in which GABA transport was assessed by following labelled GABA movement there was no net inward transport of GABA. This

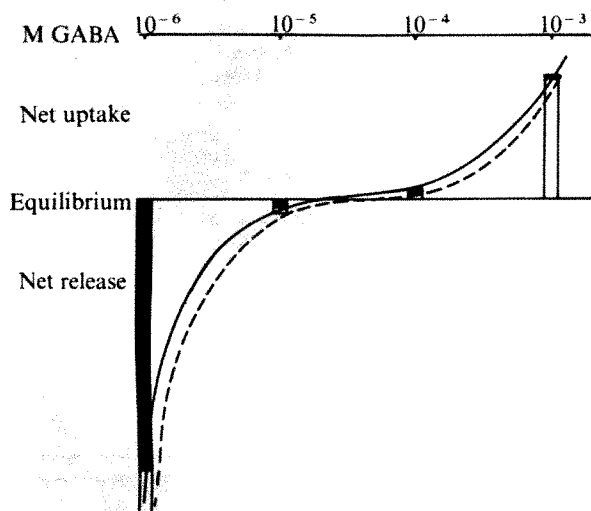


Fig. 2 Equilibrium position of GABA at a variety of initial substrate concentrations. The solid line represents incubation in normal conditions (140 mM Na^+ , 3 mM K^+) while the dashed line represents depolarising conditions (91 mM Na^+ and 52 mM K^+) and illustrates the shift of GABA equilibria effected by reducing the driving force.

situation is illustrated in Fig. 1, which shows the form of plots of velocity against substrate for net GABA movement as opposed to following the inward flux only. Figure 1 also shows that measurements of inward flux made on a reversible transport system result in an apparently increased V_{\max} and an apparently decreased K_m , for the inward flux originates at zero substrate concentration while the net flux is zero at the substrate concentration at which the inward and outward fluxes are balanced, thus shifting the curve away from the origin.

Figure 2 is a plot of the equilibrium position for GABA transport in two sets of conditions. The first involved normal uptake media while the second was carried out using a depolarising medium. The alteration in media has the effect of shifting the equilibrium position between net uptake and release. These data suggest that in normal extracellular media no mechanism exists for removing GABA much below 10^{-4} M. This apparent dilemma is resolved by considering quantitatively the factors controlling GABA equilibrium across the synaptosomal membrane. At equilibrium the ratio of internal to external GABA is equal to

$$\frac{[\text{GABA}]_i}{[\text{GABA}]_o} = \left(\frac{[\text{Na}^+]_o}{[\text{Na}^+]_i} \right)^3 \left(\frac{[\text{K}^+]_i}{[\text{K}^+]_o} \right) \exp(-2\Delta\psi F/RT)$$

This assumes that the energy for GABA transport resides entirely in the ionic gradient and that 3 Na^+ and 1 K^+ ion are translocated with each molecule of GABA moved. This stoichiometry fits our unpublished results and agrees with published studies relating GABA transport to ionic fluxes¹³. This equation accounts quantitatively for the shift in GABA equilibrium shown in Fig. 2 when a depolarising medium was used.

Such a correspondence indicates that as a first approximation the forces available in the electrochemical gradient seem to control the distribution of GABA. Because the membrane potential of isolated synaptosomes is considerably less than that of a synaptic ending *in vivo*, we would expect the point at which concentrative uptake begins to shift to lower concentrations considerably. In fact using reasonable estimates for the Na^+ and K^+ gradient and assuming a membrane potential in the range of -50 to -60 mV, it seems that there is sufficient energy in the electrochemical gradient to concentrate GABA more than 100,000-fold in a synaptic ending. From the highest estimates of GABA concentration inside a nerve ending of 100 mM (ref. 14), we can estimate that the balance point at which concentrative uptake begins would be in the range of 10^{-6} M external GABA. This suggests that "high affinity" uptake systems are functionally operative in intact nervous tissue but only detectable kinetically in isolated synaptosomal preparations, which cannot exhibit net uptake in these conditions.

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Strain-dependent variations in number of midbrain dopaminergic neurones

THE activity of tyrosine hydroxylase (TH), the enzyme catalysing the initial and presumed rate-limiting step in the biosynthesis of the catecholamine neurotransmitters dopamine (DA) and noradrenaline (NA), varies over a twofold range in the brains of different strains of inbred mice¹. The molecular mechanisms responsible for these strain-dependent differences in enzyme activity remain to be established^{1,2}.

We have examined the regional differences in TH activity in the brains of BALB/cJ and CBA/J mice. Using immunochemical and immunocytochemical methods, we have investigated: (1) if the difference in TH activity in the whole brains of the two strains is regionally discrete and localised to either the DA or NA neuronal systems; (2) if the difference in enzyme activity can be attributed to variations in either the amount or state of activation of enzyme molecules, and (3) if there are strain-dependent differences in the number of neurones containing the enzyme.

Male BALB/cJ and CBA/J mice (Jackson Laboratories) were killed at 8–10 weeks of age. Brain regions representing sites of high concentration of cell bodies and terminals of DA and NA neurones³ were removed by microdissection. The regions sampled included: (1) the region of the midbrain containing the substantia nigra and adjacent area of the interpeduncular nucleus which includes the cell bodies of the A9 and A10 neurones³ of the nigro-striatal, mesolimbic and mesocortical DA systems⁴; (2) the olfactory tubercle and caudate nucleus, in which TH is localised within DA terminals of the mesolimbic and nigro-striatal systems, respectively^{3,4}; (3) the nucleus locus coeruleus (LC), a region of NA cell bodies; and (4) the hypothalamus, which contains a large innervation of NA fibres and which could be examined by assaying for the activity of dopamine- β -hydroxylase (DBH), a specific biochemical marker of noradrenergic neurones⁵.

Each tissue was homogenised in 10–15 volumes of 5 mM phosphate buffer, pH 6.5, containing 0.2% Triton X-100. After centrifugation, the supernatant was assayed for TH and DBH activity as described before⁶. Immunochemical titration was performed by modification⁷ of the method of Feigelson and Greengard⁸, using specific antibodies for TH prepared from bovine adrenal medulla⁷. For immunocytochemical localisation of TH, four mice from each strain were perfused with 4% paraformaldehyde in phosphate buffer, pH 7.3, postfixed with picric acid-formalin, washed overnight in phosphate buffer and embedded in paraffin. Coronal sections (6 μ m) were taken through the entire brainstem. Sections at 150- μ m intervals through both the substantia nigra-A10 area and the nucleus LC were processed for immunocytochemical localisation of TH by the peroxidase-antiperoxidase method as described elsewhere⁹. The number of TH-containing cells in the midbrain DA neuronal areas (A9 and A10) and in the LC were counted by standard morphometric methods^{6,10}.

We found significant regional differences in the activity of TH in the brains of CBA/J and BALB/cJ mice (Table 1A). The differences were restricted to the DA neuronal systems of both the nigro-striatal and the mesolimbic systems: TH activity was lower by 45% in the substantia nigra-A10 area and by 25–30% in the caudate nucleus and olfactory tubercle in the CBA/J mice compared with the BALB/cJ strain. In contrast, no strain difference in TH and DBH activities were observed in the locus coeruleus or in the DBH activity in the hypothalamus (Table 1B), suggesting that the activity of TH (and DBH) in noradrenergic neurones was similar in the two strains.

We next investigated whether the strain difference in TH activity in midbrain DA neurones was due to a difference in the amount of enzyme protein. Immunochemical titration with a specific antibody to TH demonstrated that the "equivalence point" (the concentration of tissue (enzyme) which just precipitates a fixed amount of antibody and is an indirect measure of the relative amount of enzyme protein in the tissue) of the substantia nigra-A10 area from CBA/J

Table 1 Differences in enzyme activities and cell number in dopaminergic and noradrenergic neurones of two inbred mouse strains

A					Dopaminergic system: TH activity*				
Strain		Substantia Nigra		Caudate Nucleus		Olfactory Tubercle			
BALB/cJ		2.26 ± 0.07(27)		300.2 ± 5.5(28)		223.4 ± 9.0(8)			
CBA/J		1.25 ± 0.14(14)		226.4 ± 6.6(14)		162.6 ± 7.8(8)			
RATIO†		0.55 ± 0.06‡		0.76 ± 0.02‡		0.73 ± 0.04‡			
B					Noradrenergic system: TH and DBH activities§				
Strain		Nucleus locus coeruleus		Hypothalamus					
		TH		DBH		DBH			
BALB/cJ		13.5 ± 1.3(13)		0.86 ± 0.11(16)		189.0 ± 7.1(13)			
CBA/J		13.9 ± 1.0(18)		0.97 ± 0.11(10)		197.4 ± 6.4(13)			
Ratio†		1.03 ± 0.09(ns)		0.89 ± 0.11(ns)		0.96 ± 0.04‡			
C					Cell number and specific activity				
Strain		Substantia nigra		Nucleus locus coeruleus					
		Cell no. (N _t)		Specific activity		Cell no. (N _t)		Specific activity	
BALB/cJ		3,384.4 ± 71.3(8)		0.667 ± 0.020		629 ± 41(6)		0.022 ± 0.002	
CBA/J		1,664.4 ± 172.5(6)‡		0.751 ± 0.084ns		624 ± 53(4)ns		0.022 ± 0.002ns	

Numbers of animals (A and B) and nuclei (C) are given in parentheses. ns, Not significant from BALB/cJ area. Specific activity represents the calculated TH activity per cell.

*Activity expressed as nmol of DOPA formed per region of the substantia nigra-A10 per h and nmol of DOPA formed per g per h for caudate nucleus and olfactory tubercle.

†Ratio = (CBA/J)/(BALB/cJ).

‡P < 0.001.

§TH activity expressed as pmol of DOPA formed per region of locus coeruleus per h. DBH activity expressed as nmol of octopamine formed per region of locus coeruleus per h and nmol of octopamine formed per g per h for hypothalamus.

||Estimated cell number is calculated using the formula $N_t = n_s (S_t/S_s)$, where N_t is estimated total number of cells, n_s is the total number of cells counted in the sample, S_t is the total number of sections through the sample and S_s is the number of sections counted¹⁰.

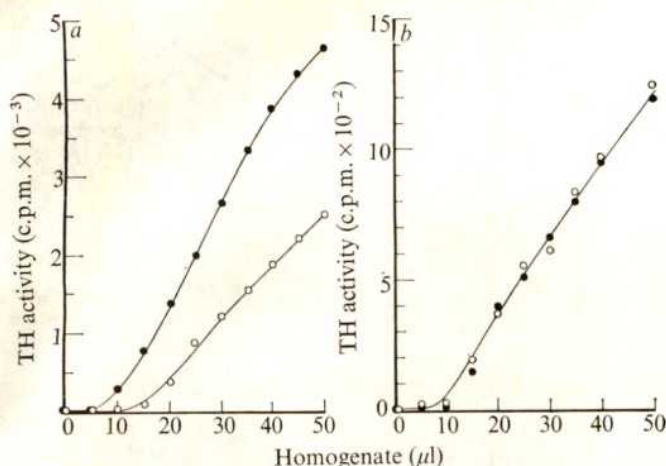


Fig. 1 Immunochemical titration of TH in the (a) substantia nigra-A10 area and (b) locus coeruleus of BALB/cJ (●) and CBA/J (○) mice. Pooled tissues from four mice of each strain were homogenised in 10 volumes (w/v) of 5 mM phosphate buffer pH 7.0, containing 0.2% Triton X-100, and centrifuged. Ten μl of antibody was added to 5–50 μl of homogenate and the final volume was adjusted to 60 μl with buffer. After incubation at room temperature for 1 h, the mixture was centrifuged and TH activity was assayed in the supernatant. In (a) the equivalence point for the substantia nigra-A10 area from the CBA/J mice (13.4 μl) is to the right of that of the BALB/cJ strain (7.0 μl). This demonstrates that the midbrain DA neurones of the CBA/J strain contain 47% less enzyme protein than the identical region of the BALB/cJ strain. The equivalence points for TH in the locus coeruleus (b) from both strains are not statistically different.

mice (13.4 μl) was situated to the right of that of the BALB/cJ strain (7.0 μl) (Fig. 1a). This indicates that the midbrain DA neurones of the CBA/J strain contain 47% less enzyme protein than the identical region of the BALB/cJ strain, which is comparable in magnitude with the difference in enzyme activity. In comparison, the equivalence points for TH of the locus coeruleus from both strains are identical (Fig. 1b), demonstrating that the noradrenergic neurones of both strains contain equivalent amounts of TH enzyme protein.

Catecholaminergic cells within the midbrain and locus coeruleus of BALB/cJ and CBA/J mice were stained immunocytochemically for TH and counted by morphometric methods. TH-containing neurones in the substantia nigra-A10 area of both strains were identified easily. In general they were similar in size, shape and intensity of staining (Fig. 2), although the neuropile of CBA/J appeared to contain more stained DA fibres. There were approximately 50% fewer TH-containing cells within the substantia nigra-A10 area of the CBA/J strain compared with the BALB/cJ mice (1,664 compared with 3,384), a difference comparable with the variation in TH activity (Table 1c). But no strain difference was evident in the calculated specific activity of TH (enzyme activity per neurone) in the midbrain DA neurones of these two strains. Thus, the strain-dependent difference in TH activity in midbrain DA neurones is not the result of a change in the specific activity of the enzyme, but a difference in the total number of DA neurones in this region.

In contrast to the DA system, the number of TH-containing neurones and the specific activity of TH in the nucleus locus coeruleus of these two strains were similar (Table 1c). It is interesting that the TH activity per cell of DA neurones is 30 times greater than that of NA neurones, substantiating the indirect evidence of other workers that the specific activity of TH is greater in DA than in NA neurones¹¹.

Our results show that the twofold difference in the activity of TH in brains of BALB/cJ and CBA/J mice¹ is explained entirely by a difference in the activity and amount

of enzyme within DA neurones. This difference in amount of enzyme protein, in turn, seems to be a consequence of a strain-dependent difference in the number of TH-containing neurones located within the substantia nigra-A10 area of the midbrain comprising the cell bodies of nigro-striatal, mesolimbic and mesocortical DA systems^{3,4}. The fact that the activity of the enzyme per neurone (that is, the specific activity) was the same in DA neurones of both strains, as were the number and specific activity of TH in the noradrenergic neurones in the locus coeruleus, makes it unlikely

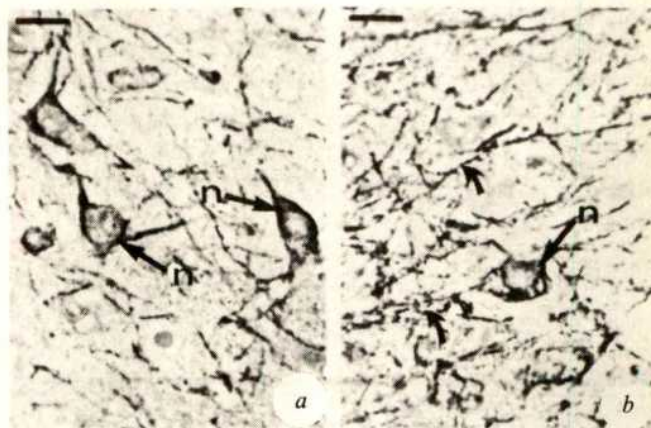


Fig. 2 Immunocytochemical localisation of TH in substantia nigra-A10 area by peroxidase-antiperoxidase method. a, In BALB/cJ strain, the neuronal perikarya (n) show selective cytoplasmic localisation of the enzyme. b In CBA/J strain, the neuronal perikarya (n) are labelled selectively for TH. The neuropile shows an especially dense plexus of labelled fibres (curved arrows) compared with BALB/cJ strain. The bars represent 50 μm.

that variations in staining could account for the observed difference in cell number. But whether the CBA/J mice have fewer DA cells, or whether such cells are present but have too little TH enzyme to be visualised by this method, cannot be answered by the techniques we used.

Although the activity of TH differs by 50% in the substantia nigra-A10 area between these two strains of mice, there is only a 25% difference in terminal fields of the caudate nucleus and olfactory tubercle. This suggests that DA neurones of CBA/J mice have more axonal ramifications per cell, serving to enlarge the terminal fields and compensate for the absolute diminution in cell number. This might also explain the observed increase in the density of TH-staining within the neuropile of the midbrain in this strain.

There is one important clinical implication of this study. It has been postulated that the onset of symptoms in several "degenerative" diseases of the brain, including Parkinson's disease, results from a reduction in the number of specific neurones below a critical number. Thus, individuals with fewer nerve cells may be more susceptible to disease than those endowed with more neurones. This study offers direct evidence that there may be marked, genetically-determined, differences in the number of neurones of a particular chemical class between groups of neurologically normal mice. That DA neuronal systems are affected raises questions about the susceptibility of individuals to Parkinsonism and other proposed diseases of DA neurones, possibly including schizophrenia¹². Thus, genetic variation in the number of neurones within chemically specific cell groups in the brain might be important in influencing the susceptibility to the expression of some diseases of brain.

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Circadian rhythm in cerebrospinal fluid noradrenaline of man and monkey

BRAIN areas with the highest concentration of noradrenergic nerve terminals and noradrenaline (NA) have a circadian rhythm in their content of NA in the rabbit, rat and cat. The anterior and lateral hypothalamus¹ and cervical spinal cord², midbrain³ and caudate nucleus⁴, medial lower brain stem⁵ and frontal cortex⁶ all have significant circadian variations in their NA content. These brain regions have their highest NA level during the night in the rat, a nocturnal animal, and during the day in the rabbit and cat.

NA is present in cerebrospinal fluid (CSF) in measurable quantities⁷⁻¹⁰ and the brain areas having the highest NA concentrations are located in close proximity to the CSF². Levels of NA in CSF are affected by uptake into these brain areas since NA injected into the lateral cerebral ventricle is actively and rapidly taken up into catecholaminergic neurones lying adjacent to the cerebral ventricles^{11,12}. NA levels in CSF should also reflect release from adjacent brain areas as the concentration of NA in brain tissues is typically 1,000 times as high as in CSF⁷. We have measured the NA content of CSF from monkey and man and found a circadian rhythm with NA content highest in the afternoon. This rhythm is persistent even during altered environmental stimulation and lighting.

NA was measured by the radioenzymatic method of Henry *et al.*¹³ as modified for CSF by Ziegler *et al.*⁷. This method enzymatically converts NA to ³H-adrenalin by the phenylethanolamine-N-methyl-transferase catalysed transfer of a ³H-methyl group from ³H-S-adenosylmethionine. The assay could detect 20 pg NA per ml of CSF.

CSF was continuously sampled from four different monkeys on a total of 34 d. The CSF flowed at a rate of 0.8 ml h⁻¹ from a chronically implanted cannula in the lateral cerebral ventricle into a fraction collector kept at 3 °C and set to advance one tube every 3 h. Each collection tube contained 10 mg ascorbic acid to prevent oxidation of NA. NA levels in CSF from the monkeys' lateral ventricle were lowest from 2400 to 0900 and then rose in the morning and plateaued from 1200 until 1500. CSF was collected from the monkeys on weekdays while researchers and technicians were working in close proximity to the animals, on weekends when the monkeys were seen for only a short period by a single person, and while confined

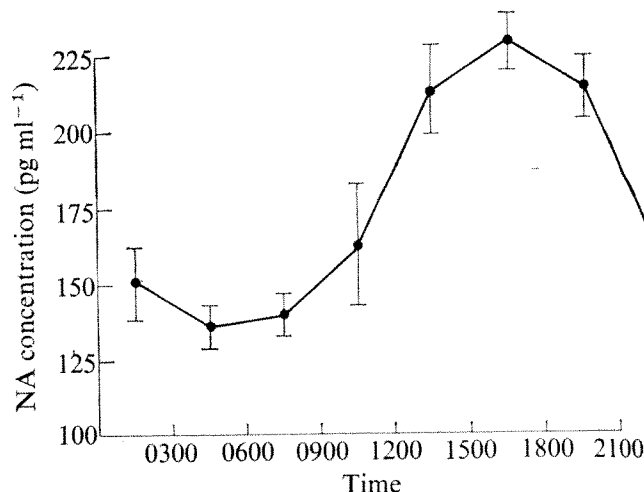
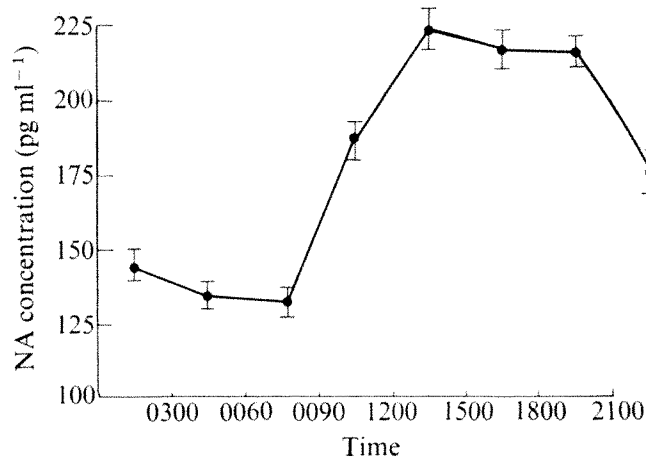


Fig. 1 Concentrations of NA in CSF collected from the lateral ventricle of 4 monkeys on 34 d. Samples were collected over 3 h periods and concentrations are shown as pg ml⁻¹ of NA \pm s.e.m.

to a closed box which was illuminated from 0600 to 1800. Feeding conditions remained constant throughout the entire study with the animals being manually fed only Purina monkey chow no. 5038 twice daily at 0830 and 1330, 7 d a week. Under these three different conditions there was no discernible difference in the circadian rhythm of CSF NA. The mean NA levels in CSF from these monkeys are shown in Fig. 1. One monkey was confined to a box that was dark from 0600 to 1800 and light during the night for 18 d. His CSF NA levels were measured on nine of those days and maintained the same circadian pattern (Fig. 2) and did not detectably vary from the beginning to the end of this reversed light cycle period. The eight means (one from each collection time period) from the regular light cycle (light from 0600 to 1800) were treated as a single vector as were the eight means for the reversed light cycle. The comparison of the regular and reversed mean vectors was made using Hotelling's *t* square test. The *F* value was not significant, *P*=0.18. Even when individual Student *t* tests were performed on each pair of the eight means, no significant differences were encountered.

Twenty-one patients hospitalised at NIH for various neurological disorders had CSF sampled by lumbar puncture for diagnostic purposes. All the patients were confined to strict bed rest for 18 h before lumbar puncture

Fig. 2 Concentrations of NA in the CSF of one monkey kept in an isolation box that was dark from 0600 to 1800 and light during the night. Samples were collected during the first 3 and last 6 d of an 18 d period and values are pg ml⁻¹ of NA \pm s.e.m. of all samples collected during this reversed light cycle.



which was performed at 0300, 0900 or 1500. The patients' physician collected the 16th to 20th ml of CSF removed into a polypropylene tube containing 10 mg ascorbic acid and placed this tube on ice until it was transferred within 30 min to a -70°C freezer for storage. We have previously shown that there is a gradient of increasing NA in the first 12 ml of lumbar CSF removed from adults⁷ but that NA levels do not further increase as more CSF is removed (C.R.L., J.H.W., M.G.Z. *et al.*, unpublished). NA levels in human CSF are significantly lower at 0300 than at 0900 or 1500 ($P < 0.005$, Student *t* test), but the 0900 and 1500 NA levels do not significantly differ (Fig. 3). Several of the patients in this study had seizure disorders and were receiving phenobarbital, phenytoin and ethosuximide; these drugs do not seem to alter levels of NA in CSF. The patients with seizure disorders are randomly distributed throughout the three groups of patients (Table 1). The levels of NA in human CSF seem to parallel monkey NA levels both in concentration of NA and the direction of the circadian rhythm.

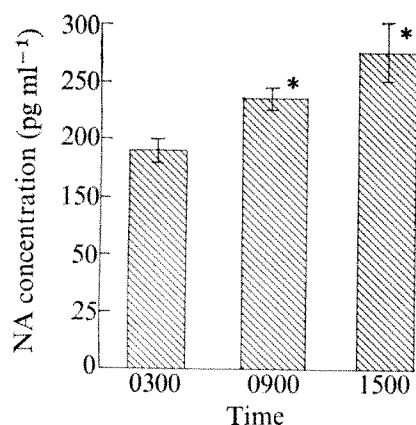


Fig. 3 Levels of NA in the CSF of human patients at 0300, 0900 and 1500. The NA levels of CSF of individual patients are given in Table 1. *Significantly higher than the NA level at 0300 ($P < 0.005$ by Student's *t* test).

NA levels in CSF must represent the amount of NA released into the CSF minus the amount cleared by uptake or chemical conversion. There are several reasons why variations in neuronal release of NA might be the cause of the cyclic variations of NA in the CSF. Levels of NA are highest in CSF during the period when primates are most active, and in depressed patients activity increases the excretion of 3-methoxy-4-hydroxyphenylglycol (MHPG)¹⁴ the major central nervous system metabolite of NA. Noradrenergic nerve terminals in brain areas adjacent to the CSF have their highest NA content in the cat, rat and rabbit when these animals are usually awake¹⁻⁶. Drugs that block NA synthesis or deplete brain NA decrease motor activity¹⁵ and drugs that release NA increase the rate of performance of conditioned behaviour¹⁶. The infusion of NA into the cerebral ventricles increases locomotor activity¹⁷. It seems reasonable that the higher level of NA in CSF of primates during their most active part of the day represents an increased rate of release of NA from nerve terminals. The rhythm of NA in CSF probably does not represent NA synthesis in the entire brain as both the NA content¹⁸ and rate of NA synthesis¹⁹ are highest in rat whole brain during the time of day when rats usually sleep and are least active.

The NA rhythm of the pineal gland is altered by environmental lighting²⁰ but the NA rhythm in the cervical cord and anterior hypothalamus is not changed by an altered lighting schedule³ and is endogenous to the animal.

Table 1 Age, diagnosis, time of lumbar puncture and CSF level of NA in 21 patients

Patient	Age (yr)	Diagnosis	Time	NA in CSF
A	60	Choroid plexus papilloma	0800	210
B	51	Occipital lobe AVM*	0300	182
C	29	Seizures	0300	146
D	18	Seizures	0300	201
E	23	Seizures	0300	203
F	21	Spinal cord AVM*	0300	205
G	67	Cervical spondylosis	0900	234
H	73	Spinal cord AVM*	0900	244
I	57	Temporo-occipital AVM*	0900	212
J	32	Seizures	0900	261
K	51	Extracranial AVM*	0900	274
L	23	Pituitary tumour	0900	225
M	29	Spinal cord AVM*	0900	210
N	63	Dizziness	0900	226
O	16	Seizures	1500	223
P	26	Scalp infection	1500	270
Q	27	Seizures	1500	206
R	36	Seizures	1500	373
S	31	Seizures	1500	325
T	32	Seizures	1500	208
U	48	Headache	1500	326

*AVM = arterio-venous malformation

The circadian rhythm of NA in monkey CSF was not altered by 18 d of a change in light cycle analogous to travel to a point half-way around the world and is apparently endogenous to the animal.

Some drugs that interact with brain NA have variable effects in the rat depending on the time of day they are administered. Reserpine depletes brain NA most effectively in the dark and the mortality rate from reserpine varies with the time of administration in rats²¹. Methamphetamine improves task performance in rats best in the dark¹⁶. These drugs might have their most marked central nervous system effects in man during the day.

Several investigators have measured NA in CSF by fluorometric assays and reported increased levels of NA in patients with stroke²², hypertension²³ and psychiatric disorders⁹. Others have reported abnormal levels of MHPG, the major brain metabolite of NA, in the CSF of patients with depression²⁴, mania²⁵ and schizophrenia²⁶. Further studies of CSF levels of NA and its metabolites should be controlled for time of sampling and activity and in man might best be done in the afternoon when NA levels seem to be relatively constant.

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Motor endplate alterations in schizophrenic patients

NEUROMUSCULAR pathology has been described in a variety of mentally ill patients, primarily those suffering from schizophrenia or the affective psychosis. Some psychotic patients have increased electrical activity of skeletal muscle at rest¹, abnormal electromyograms², perform poorly on various tests of neuromuscular coordination³, have elevated serum creatine phosphokinase activity during an acute psychotic episode⁴ and show an increased incidence of morphological abnormalities of skeletal muscle^{5,6}. Such studies have suggested that there may be an alteration of muscle innervation in such patients. We have previously reported that psychotic patients have an increased incidence of branching and sprouting of intramuscular nerve twigs⁶. We now report significant alterations in the morphology of the nerve terminals at the motor endplate in psychotic patients. This suggests that there may be an abnormality of transmission at neuromuscular synapses in some psychotic patients.

Subjects were 24 in-patients at a psychiatric unit at the Illinois State Psychiatric Institute. Eight were men and 16 were women: their mean age was 28, with a range of 19-42. Each patient was evaluated by two psychiatrists and a psychiatric social worker, and a research diagnosis was established by consensus. All subjects met the criteria for a diagnosis of schizophrenia on the Yale-New Haven Schizophrenia Index. Patient data were compared with results obtained from five normal, healthy volunteers who had no personal or family history of mental illness.

Muscle biopsies of the peroneus brevis muscle were performed on all subjects as described previously⁶. Peroneus brevis was chosen as the biopsy site because the muscle is relatively easily biopsied and there are detailed analyses in the literature of the pattern of methylene blue stained intramuscular nerve endings in this muscle^{7,8}. The subterminal motor neurones were supravitaly stained with methylene blue⁹ and the motor neurone terminal structure analysed according to the methods of Allen et al.¹⁰. Twenty-five to fifty randomly selected nerve terminals from each subject were either photographed or traced with a camera lucida apparatus, enlarged photographically, and measured with a planimeter. Parameters studied were: the

number of terminal bulbs per endplate, N ; the area of the smallest rectangle which could be fitted to the endplate region, A_T ; the total cross-sectional area of the terminal bulbs, A_N ; the mean terminal bulb area, A_N/N ; and the ratio of A_N to A_T or the synaptic index, S.I.

The differences in nerve terminal organisation of patients from those of controls are represented in Fig. 1 and summarised in Table 1. The number of terminal bulbs per endplate, N , was not significantly different between patients and controls. But the size and dispersion of the terminal bulbs was significantly altered in psychotic patients. The mean total area of endplate arborisation, A_T , was both larger and more variable in patients than controls. Similarly, the patient endplate bulb area, A_N , was significantly more variable. The tendency toward a smaller endplate bulb area of greater variability in patients was demonstrated by the significantly smaller average cross-sectional area A_N/N in the patient group. The S.I., a measure of the density of neural structures at the endplate is significantly lower in patients than controls, and more variable ($F = 31.48$, $P = 0.008$).

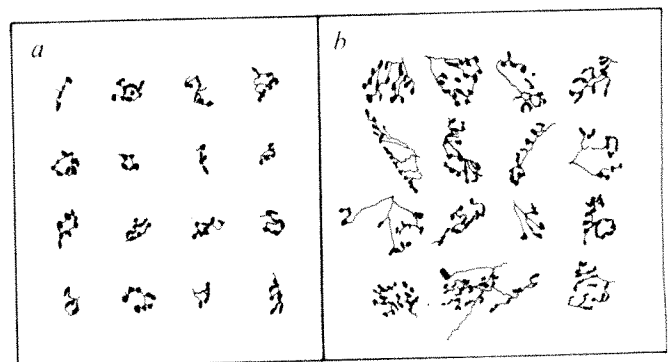


Fig. 1 Comparison of methylene blue-stained motor neurone terminals in muscle biopsy specimens from a normal volunteer control (a) and a schizophrenic patient (b). Biopsies were taken from peroneus brevis muscle.

We also studied the relationship of subterminal nerve branching as assessed by the functional terminal innervation ratio (FTIR) to the terminal arborisation parameters. Figure 2 illustrates a motor neurone from a psychotic patient having extensive branching and increased elaboration of the motor nerve terminals at the endplate. We explored the possibility that only endings on branched neurones would show the endplate alteration, but this did not seem to be the case. In a given patient, if the endplate region was distorted, most endings were affected regardless of whether they belonged to branched or unbranched neurones. The Pearson product-moment correlation coefficient, r , for the relationship between the FTIR and both A_N and A_N/N were significant ($r = 0.3607$, $P = 0.038$; $r = 0.4385$, $P = 0.014$ respectively). Similarly, the FTIR was significantly correlated with A_T ($r = 0.3490$, $P = 0.044$). Surprisingly, the FTIR did not show a significant correlation with the S.I. Closer examination of the data revealed that this result was obtained because both A_N and A_T increased as the amount of branching increased; therefore, the ratio A_N/A_T tended to remain constant as FTIR increased. There was, however, the expected trend towards a negative correlation between increased FTIR and reduced S.I.

There was no significant difference between diagnostic subtypes such as paranoid and non-paranoid, or acute schizophrenic and chronic schizophrenic, with respect to the parameters studied. In our previous work, we have found muscle pathology in manic-depressive psychotic patients as well as schizophrenic patients. Preliminary observations of three manic-depressive patients suggests that two of three patients have endplate enlargement with a reduced S.I. There was no significant correlation between endplate architecture and length of illness, amount or type of psychotropic medication received, or duration of current illness. The endplate abnormalities were

Table 1 Comparisons of control and psychiatric patient endplate histometric parameters

Variable	Control	Patient	P (t test)
N	$6.72 \pm 1.14^*$	7.48 ± 1.31	0.242
$A_T (\mu^2)$	2.53 ± 0.47	3.33 ± 1.70	0.055
$A_N (\mu^2)$	0.96 ± 0.07	0.86 ± 0.38	0.211
$A_N/N (\mu^2)$	0.14 ± 0.01	0.11 ± 0.04	0.005
S.I.	0.40 ± 0.05	0.28 ± 0.06	0.001
FTIR	1.11 ± 0.05	1.40 ± 0.32	0.001

*Mean \pm s.d.

not age or sex related. The fact that only one of our subjects was over 40 yr of age would tend to rule out the possibility that changes in synaptic indices were due to ageing, since all previously described age changes in neuromuscular structure begin to occur only after the sixth decade of life¹¹.

Nonspecific nerve injury or trauma is a possibility to be considered. There are no previous descriptions of endplate reactions such as those described here to trauma. There are descriptions of smaller terminal arborisations of regenerating neurones in non-psychotic subjects¹². Furthermore, an increased incidence of muscle fibre abnormalities, such as scattered atrophy and fibre type grouping has been seen in biopsies of more proximal muscles in psychotic patients (such as biceps brachii and vastus lateralis), which might be expected to show fewer nonspecific signs of neuronal degeneration in response to age or trauma^{13,14}.

Thus, the endplate region in psychotic patients tends to be larger, and with less nerve terminal area per unit of endplate area. This pattern resembles changes described in myotonic dystrophy¹⁰ and the neuropathy associated in some patients with coeliac disease¹⁵. Since our patients also had increased collateral branching of motor neurones, the possibility exists that the terminal alterations described here are related to the process of regeneration by which motor neurones restore function to previously denervated fibres. This would suggest that psychosis is associated with a neuropathic process with destruction of terminal motor neurones followed by compensatory structural alterations.

It seems likely that the structural alterations noted here are associated with some functional abnormality, although no relevant studies of neuromuscular transmission in psychotic patients have as yet been reported. Kuno *et al.*¹⁶ have shown that spontaneous and evoked acetylcholine (ACh) release at the neuromuscular synapse is proportional to the size of the endplate area. Since endplates in psychotic patients tend to be larger than in normals, ACh release might be expected to be greater than normal. However, the total area of the terminal bulbs, A_N , is smaller in the patients. Since the resultant decrease

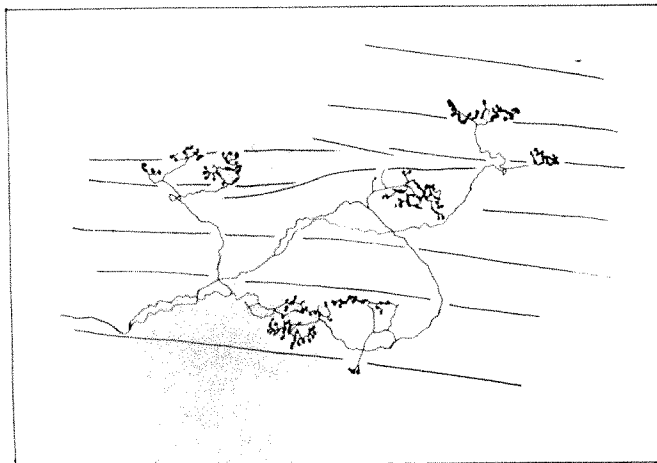


Fig. 2 Extensive terminal branching of a motor neurone in a muscle biopsy specimen from a schizophrenic patient. Normally, the intramuscular nerve ending does not branch, innervating only one muscle fibre. Here, eight muscle fibres are innervated by the single neurone. The endings show increased elaboration and enlargement.

in terminal bulb surface area is proportional to the square of the radius of the bulb, there is a proportionately greater reduction in surface area than is represented by the reduction in cross-sectional area of the bulbs. These considerations suggest that spontaneous or evoked ACh release at the neuromuscular synapse in psychotic patients may be abnormal, but the pattern of abnormality would be difficult to predict *a priori*. Conceivably, the abnormal pattern could contribute to the development of pathological morphology in skeletal muscle fibres.

The significance of these findings for our understanding of the psychotic process remains to be elucidated. The abnormalities in the subterminal nerve may be a reflection of altered central nervous system physiology such as the putative defect in dopamine metabolism thought to be present in schizophrenic illness. We have recently found (unpublished) that motor neurone excitability as measured by spinal monosynaptic reflex testing is altered in some psychotic patients. The excitability changes are interpretable in terms of altered, descending supraspinal influences. A second possible way to relate these findings to the pathophysiology of psychosis is that the altered motor neurone terminals may reflect a widespread neuronal abnormality that affects neurones in many parts of the nervous system. In either case, the neuromuscular junction seems to be a relatively accessible and potentially rewarding site for studying synaptic neurophysiology in the mentally ill.

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Brain and retinal damage from lathyrus excitotoxin, β -N-oxalyl-L- α , β -diaminopropionic acid

HUMAN consumption of lathyrus peas, especially during periods of famine, has long been associated in certain parts of the world with outbreaks of neurolathyrism, a crippling neurological disorder characterised by spastic paraplegia¹. A neuroexcitatory² amino acid, β -N-oxalyl-L- α , β -diaminopropionic acid (ODAP), isolated from the seeds of *Lathyrus sativus*³ is suspected to be the responsible neurotoxic principle. Although paralysis of the lower extremities has been reported in adult monkeys after lumbar intrathecal administration of ODAP⁴, and similar symptoms have been induced in newborn chicks by intra-peritoneal administration⁵, evidence for central nervous tissue damage from systemically administered ODAP has been lacking. We now report that ODAP, administered intra-peritoneally to immature mice, induces lesions in the retina, hypothalamus and lower medulla. This pattern of damage is similar to that demonstrated in animals after oral or subcutaneous administration of glutamate (Glu).

ODAP resembles Glu in molecular structure and is a neuro-excitant² and convulsant⁶ similar to but more potent than Glu. When given either subcutaneously⁶ or orally⁷ to infant rodents,

Glu rapidly destroys nerve cells in the retina or select regions of the brain. Several years ago we explored the molecular specificity of this phenomenon^{8,9} and found that structural analogues of Glu known to have neuroexcitatory properties^{10,11}, including aspartic, cysteic, homocysteic, *N*-methyl aspartic and kainic acids, reproduced the Glu type of neuropathological syndrome; analogues known to be more potent than Glu as neuroexcitants were also more potent as neurotoxins. ODAP was not available for inclusion in our earlier studies but was subsequently synthesised by one of us (C.H.M.).

ODAP was synthesised from the copper chelate of L- α , β -diaminopropionic acid and ethyl oxalyl chloride by the method of Rao *et al.*³. The product melted at 206 °C (at which it decomposes), and was free from impurities as evidenced by homogeneity in high voltage electrophoresis at pH 3.5. Elemental analysis showed our final product to be the monohydrate of β -*N*-oxalyl-L- α , β -diaminopropionic acid. The analysis calculated for $C_5H_8N_2O_5 \cdot H_2O$ was C, 30.93; H, 5.15; N, 14.43: that found was C, 30.86; H, 5.28; N, 14.34.

Infant Swiss albino mice 7–8 d old were used. Pilot experiments showed that mice in this age range could tolerate intraperitoneal doses of ODAP up to 0.35 mg per g body weight but tended to convulse to death at higher doses. ODAP was administered intraperitoneally at 0.35 mg per g body weight to 56 infants from 8 litters. Controls consisted of two animals from each litter given an equimolar dose of sodium chloride. All animals were killed under anaesthesia by perfusion fixation 3–5 h after treatment and prepared for combined light and electron microscopic examination of the retina and brain as described elsewhere¹².

ODAP caused a lesion in the retina of infant mice (Fig. 1a) which was very similar, if not identical, to the lesion seen after treatment with Glu¹³. In the hypothalamus, the arcuate nucleus is affected preferentially by Glu, and the typical effect, illustrated extensively elsewhere^{7,8,12}, consists of acute swelling of dendrites and cell bodies of neurones, with degenerative changes occurring rapidly in both intra-cytoplasmic organelle systems and neuronal nuclei. Axons terminating in or passing

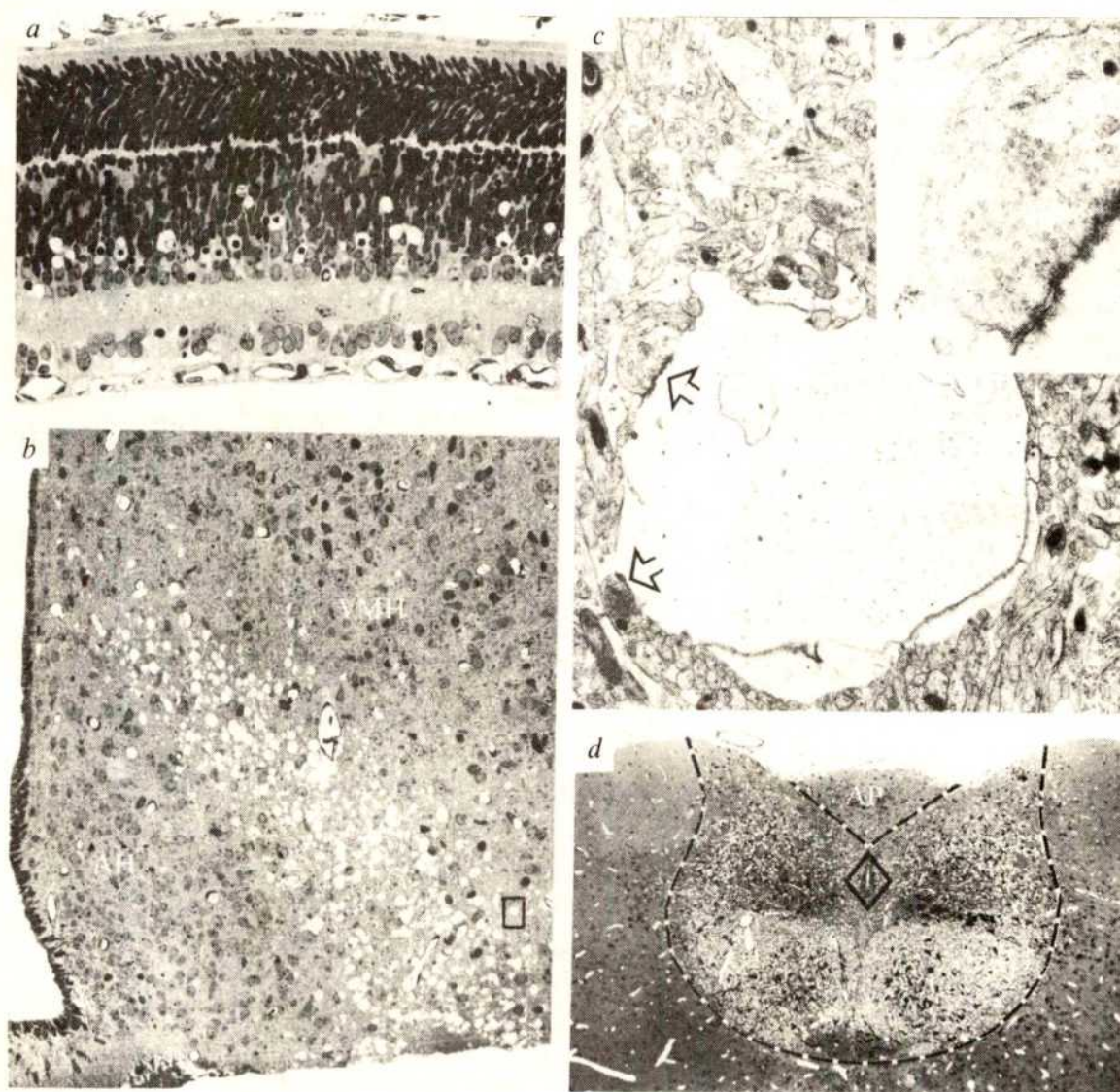


Fig. 1 *a*, A light micrograph of the retina of an 8-d-old mouse 5 h after treatment with ODAP. Acutely necrotic neurones (bull's eye profiles) in bipolar, amacrine and ganglion cell layers are evident, as are dilated dendritic inner plexiform processes ($\times 140$). *b*, A survey electron micrograph of the arcuate (AH)-ventromedial (VMH) hypothalamus of an ODAP-treated neonatal mouse, depicting a band of dilated dendritic processes interposed between arcuate and ventromedial nuclear zones. Scattered ventromedial nucleus neurones appear as dark shrunken profiles. *c*, Swollen, degenerate process from boxed region in (*b*) which is identified as a dendrite by the two synaptic terminals (arrows) impinging on its surface ($\times 8,000$). Inset, magnified view of synapse at upper arrow ($\times 22,000$). *d*, Light micrograph of section at brain stem-spinal cord junction to illustrate the extent and pattern of tissue reaction to ODAP. The area postrema (AP) is relatively unaffected but other regions within a chalice-shaped zone (broken outline) about the central canal (marked by a diamond) are damaged. Numerous oedematous dendritic processes give the lesion a rarefied appearance against which dark, shrunken neuronal cell bodies stand out. The latter are also present in scattered distribution beyond the margins of the oedematous zone ($\times 40$).

through the arcuate nucleus are characteristically spared. Treatment with ODAP resulted in acute histopathological changes in the general vicinity of the arcuate nucleus but the pattern of lesions was different from that associated with Glu treatment. ODAP induced marked swelling of a regionally distinct band of dendritic processes confined to the cell-poor zone between the ventromedial nucleus and arcuate nucleus but had little or no effect on tissue components within the arcuate nucleus proper (Fig. 1b). Processes affected by ODAP were identified as dendritic on the basis of axodendritic synaptic boutons impinging on their surfaces (Fig. 1c). The toxic reaction was restricted to the dendritic (postsynaptic) component of the synaptic complex with sparing of the presynaptic (axonal) component. Dark cell changes, a phenomenon often encountered as a minor feature of Glu neurotoxicity, was relatively more pronounced in the ODAP reaction and the cell bodies affected in the hypothalamus were almost exclusively neurones of the ventromedial nucleus. Although the significance of such changes remains obscure, it is interesting that they were a consistent concomitant of ODAP treatment but were not observed in controls.

Degenerative changes have been observed in the area postrema after oral¹⁴ or subcutaneous¹⁵ administration of Glu to infant mice. The brain stem of mice treated with ODAP in this study was damaged at the level of the area postrema and the extent of damage to the brain stem was relatively greater for any given animal than the damage sustained in the hypothalamus. Typically the lesion induced in the area postrema by Glu is confined to neural elements within the area postrema proper. The pattern of damage induced by ODAP was different; the reaction began at the margins of the area postrema, spared the area postrema proper and tended to spread caudally down the spinal cord in a circular pattern about the central canal (Fig. 1d). A spreading lesion by ODAP at this level in the neuraxis might account for the spastic paraplegic manifestations associated with human ingestion of lathyrus peas containing ODAP. Accordingly, the lesion induced by ODAP at the cervical spinal level warrants more detailed neuropathological investigation.

Here we have shown that systemically administered ODAP has potential for damaging the tissue of the central nervous system. The pattern of distribution of lesions (retina, arcuate nucleus and area postrema) and the dendritic (postsynaptic) locus of the toxic interaction makes the ODAP reaction sufficiently similar to Glu neurotoxicity to consider ODAP an excitotoxic amino acid, that is, one of the structural analogues of Glu which possesses both the neuroexcitatory and neurotoxic properties of Glu and which, therefore, may owe its neurotoxic activity to its neuroexcitatory properties.

ODAP [$\text{CO}_2\text{H}-\text{CO}-\text{NH}-\text{CH}_2-\text{CH}(\text{NH}_2)-\text{CO}_2\text{H}$] is not as closely related in molecular structure to Glu [$\text{CO}_2\text{H}-\text{CH}_2-\text{CH}(\text{NH}_2)-\text{CO}_2\text{H}$] as are other straight chain excitotoxic amino acids. This may account for certain differences noted between the patterns of lesions caused by ODAP and Glu. Whether such differences reflect dissimilar processing of the two compounds by blood brain barriers or differential binding at receptors within affected brain regions awaits further study. The conformational constraints of the ODAP molecule, however, might be such as to cause it to interact more selectively than Glu at central excitatory receptor sites. It is also possible that other species of receptors, for example high-affinity uptake receptors which may serve a protective function in brain¹⁶, respond differentially to ODAP and Glu. Balcar and Johnston¹⁷ have reported evidence suggesting that ODAP has little or no affinity for receptors of the Glu high-affinity uptake system. All such factors, as well as species, age and nutritional differences between famine-stricken man and the healthy neonatal mouse—plus individual differences among humans, especially in blood brain barrier characteristics—must be taken into consideration in attempting to understand how ODAP causes neurolathyrism. Whether ODAP, like Glu^{7,14}, can damage the brain when taken orally should also be ascertained.

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Persistence of prolonged light-induced conductance change in arthropod photoreceptors on recovery from anoxia

FOR a wide variety of photoreceptor cells, it has been shown that light alters the conductance of the cell membrane, giving rise to the voltage response of the cell^{1–4}. It is generally accepted that the primary event in the photo-excitation process is a light-induced isomerisation of the photopigment chromophore. The mechanisms linking this isomerisation to the conductance change remain unknown. It has been suggested recently that the transduction process may depend on oxidative metabolism. Intracellular recordings from photoreceptors of the honeybee (*Apis*)⁵, horseshoe crab (*Limulus*)⁶ and barnacle (*Balanus*)—observed recently by one of the authors (A.M.) in collaboration with R. Lantz—have shown that the light-induced conductance increase during illumination can be reversibly blocked by anoxia. Neither the site nor the mechanism of the action of anoxia on the transduction process is known. The light-induced spectral transitions of the photopigments are known, however, to take place under a variety of conditions including anoxia^{6–10}.

In photoreceptors of many arthropods, an intense, coloured stimulus is known to induce a prolonged depolarisation which far outlasts the stimulus^{11–14}. This phenomenon has been termed the prolonged depolarising afterpotential (PDA). In the compound eye of *Drosophila*, an intense blue stimulus ($\lambda=470\text{ nm}$) which shifts a substantial fraction of the photopigment, rhodopsin, ($\lambda_{\text{max}}=480\text{ nm}$)¹⁵, to its thermally stable photoproduct, metarhodopsin, ($\lambda_{\text{max}}=580\text{ nm}$), induces the PDA¹⁶. The PDA so induced decays rapidly if metarhodopsin is photoregenerated to rhodopsin. In *Balanus*, the ionic mechanisms underlying this prolonged afterpotential have been found to be the same as those underlying the normal, light-coincident photoreceptor voltage response¹⁷. Furthermore, from the analysis of the voltage noise superposed on the photoreceptor potential of a *Drosophila* mutant, it has been suggested that the PDA is produced by a summation of a large number of discrete potential fluctuations, just as the light-coincident receptor potential is¹⁸. In this work we studied the effect of oxidative metabolism on the PDA phenomenon in the hope that we might gain some insight into the excitation process in photoreceptors.

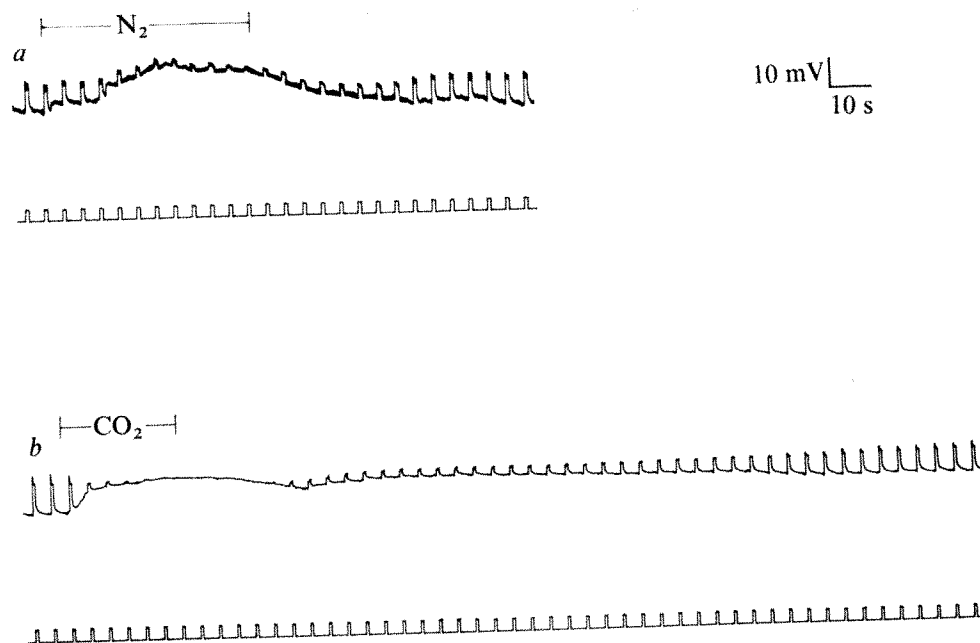


Fig. 1 In these intracellular records, the stimuli were steps of orange light ($\lambda = 600$ nm) at the highest available intensity. For experiments shown in this and the following figures, the light source consisted of a 150-W xenon arc lamp and a Bausch and Lomb high-intensity monochromator. The illuminance at the level of the preparation was about 3×10^{14} photon $\text{cm}^{-2} \text{s}^{-1}$ at both 470 nm and 600 nm. *a*, Effect of N_2 ; *b*, response when CO_2 was used. In both cases, the membrane started to depolarise and the photoresponse diminished soon after the introduction of N_2 or CO_2 . The time course of recovery is different for the two cases. The resting potential of this cell was 27 mV. The horizontal lines indicating the time when N_2 or CO_2 was applied also indicate the zero level for the potential.

Our experiments consisted of intracellular and extracellular recordings from intact preparations of the white-eyed strains of *Drosophila*. Techniques for both intracellular¹⁹ and extracellular²⁰ recordings have been described in detail elsewhere. The animal was placed inside a partially

sealed chamber and either N_2 or CO_2 was allowed to flow into the chamber, lowering the O_2 tension inside the chamber by displacement. The N_2 or CO_2 exerted its effect apparently through the respiration of the animal.

Figure 1 shows the effect of N_2 or CO_2 on the voltage

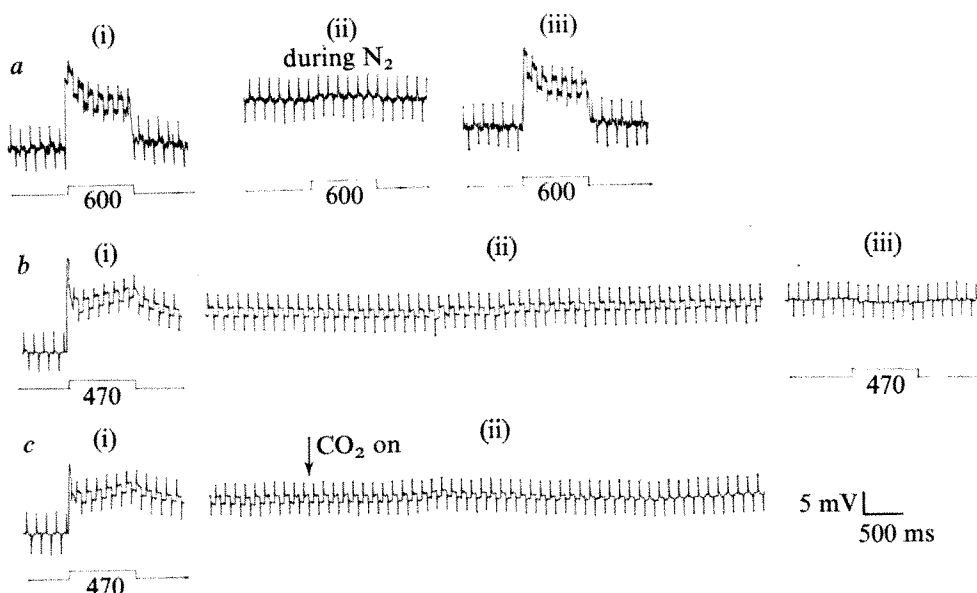


Fig. 2 Intracellular recordings: the conductance was measured by applying current pulses (0.1 nA) to the cell and recording the voltage response of the membrane with a bridge circuit. The bridge was balanced each time before the onset of light. *a*, Trace (i) shows the conductance increase due to a step stimulus of orange light ($\lambda = 600$ nm). The conductance increased only during the stimulus. Trace (ii) shows that during anoxia there was virtually no light-induced conductance change. Trace (iii) shows that the response returned to normal after the animal had recovered from anoxia. *b*, Response to intense blue light. In trace (i), the cell had been previously adapted with orange light. The conductance increased during the stimulus ($\lambda = 470$ nm) but it did not return to the dark value after the stimulus was turned off. This sustained conductance increase is the underlying mechanism for the sustained voltage response (PDA). The beginning of trace (ii) corresponds to 2 s after the PDA was fully induced. Trace (iii) shows the response to a step of blue light during PDA. There was no further increase in conductance change, consistent with the observation that a blue light causes no further depolarising voltage response during PDA. It should be noted that in this trace the bridge was balanced to the conductance during PDA and not to the dark resting value. *c*, We repeated the experiment shown in *b* except that CO_2 was introduced during PDA. The arrow marks the time when CO_2 was introduced. It can be seen that the PDA-associated conductance increase diminished gradually, and after 5 s it returned to the initial resting value (see trace (i)). Comparing trace *c* (ii) with trace *b* (ii), it can be seen that CO_2 abolished the conductance increase. When the cell fully recovered from the effects of CO_2 , no conductance increase could be elicited by a blue stimulus (identical to trace *b* (iii)) showing that the PDA effect had persisted through the period of CO_2 .

response to light. The resting membrane potential started to depolarise soon after N_2 or CO_2 was introduced, and the voltage response to light began to diminish until it was almost completely blocked. The photoresponse returned quickly after N_2 was turned off, and it recovered to its normal value in less than a minute (Fig. 1a). Figure 1b shows the response when CO_2 was used. The general features were the same as those caused by N_2 , but the recovery time of the cell was considerably longer, suggesting that CO_2 might have an effect on the conductance change in addition to that attributable to anoxia. For example, CO_2 might alter the internal pH of the cell which in turn may affect the photoresponse²¹. Once the cell had fully recovered, we could repeat the experiment many times as long as the recording situation was stable. Similar effects of N_2 and CO_2 were also observed in conductance measurements. Figure 2a(i) shows the conductance increase during the stimulus in the absence of N_2 or CO_2 . During anoxia, there was virtually no light-induced conductance change (Fig. 2a(ii)). The response returned to normal after the animal had recovered from anoxia (Fig. 2a(iii)). This suggests that the light-induced conductance change was blocked during anoxia.

Figure 2a(i) also shows that in the cell that had been previously adapted to orange ($\lambda = 600$ nm) or white light, an orange stimulus caused the conductance to increase only during the stimulus. If, on the other hand, an orange- or white-adapted cell was exposed to an intense blue stimulus ($\lambda = 470$ nm), which caused a substantial net shift of rhodopsin to metarhodopsin, the conductance increased not only during the stimulus but also long after the stimulus was turned off (Fig. 2b(i) and (ii)). This sustained conductance increase is the underlying mechanism for the sustained depolarisation (PDA). The sustained depolarisation lasted more than 30 min after the stimulus was turned off. As seen in Fig. 2b(iii), a blue stimulus caused no further increase in conductance during PDA. It should be noted that in this trace the bridge was balanced to the conductance during PDA and not to the initial dark resting value. The conductance increase during PDA was also sensitive to anoxia and CO_2 . Figure 2c(ii) shows that the conductance increase during PDA, as indicated by the bridge imbalance at the beginning of this trace, diminished gradually after CO_2 was introduced and returned to the initial resting value (see Fig. 2c(i)), 5 s after CO_2 was introduced. Comparing Fig. 2c(ii) with Fig. 2b(ii), it can be seen that CO_2 abolished the conductance increase during PDA. When the cell fully recovered from the effects of CO_2 , it was found that the PDA effect had persisted throughout the period of CO_2 . A blue stimulus was unable to produce a conductance increase just as if CO_2 had not interrupted the conductance increase due to PDA.

In muscoid diptera compound eyes, the maintained component of the electroretinogram (ERG) has been shown to originate primarily from ionic activities of the photoreceptor cells²². Accordingly, most of our experiments requiring stable, long-term recordings were carried out using the ERG. Figure 3a shows the ERG responses to intense, coloured stimuli (470 or 600 nm). If the eye was previously exposed to an intense orange light (Fig. 3a(i)), the response to the first pulse of blue light did not decay (the PDA phenomenon, Fig. 3a(ii)). Subsequent pulses of blue light elicited small responses superposed on the PDA (Fig. 3a(ii)). These superposed responses were previously shown to come from a small population of cells having spectral mechanisms different from the much larger population of cells producing the PDA¹⁸. The first response to an orange stimulus delivered during the PDA is small (Fig. 3a(iii)), but the response recovers in the subsequent pulses of orange light until its recovery is complete in the fourth pulse (Fig. 3a(iii)). The above phenomenon is caused by the reversal of the PDA effect by orange stimuli, which cause

substantial net conversion of metarhodopsin back to rhodopsin. Figure 3b and c show that the above pattern of response to a sequence of orange and blue stimuli is unchanged even if some of the stimuli are applied during the period of anoxia. Figure 3b shows the pattern of ERG responses when the light-induced voltage response was completely blocked by anoxia during the time in which blue pulses were applied. The animal was then allowed to recover from anoxia and was exposed to several pulses of orange light. It may be seen that the responses to orange pulses are small initially but recover in subsequent pulses just as in the case of the control experiment (Fig. 3a(iii)). Intracellular recordings showed that the membrane was in a high conductance (PDA) state when the cell first recovered

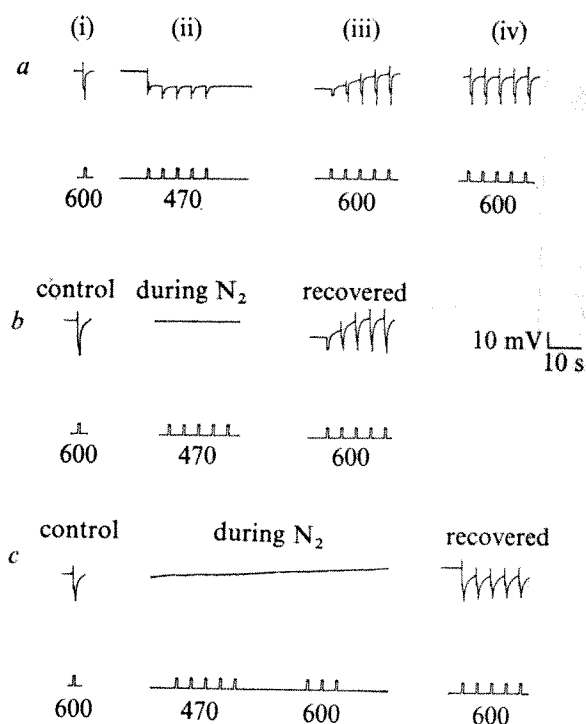


Fig. 3 a, This set of extracellular records shows the ERG responses to intense, blue or orange, stimuli (470 or 600 nm). Trace (i) shows the response to orange light. Trace (ii) shows the response to blue light when the eye was previously adapted to orange light. Note that the first pulse of blue light elicited the PDA. Subsequent pulses of blue light elicited small responses superposed on the PDA. Those responses were previously shown to come from a small population of cells that have a spectral mechanism different from the cells producing the PDA. (See text.) Trace (iii) shows the response to orange light when the cell had been blue adapted (during PDA). The first response to an orange stimulus is small. The response recovers in the subsequent pulses of orange light until its recovery is complete by the fourth pulse. Trace (iv) shows the response to orange light if the cell had been orange-adapted. (Same as trace (i)). Note that the response pattern shown in trace (iv) is very different from trace (iii). These two traces serve as a control for Fig. 3b and c. b, The introduction of N_2 blocked the light-induced response. The blue pulses induced no measurable response while the animal was in the anoxic state. After the animal had recovered, its responses to the orange stimuli showed the same pattern as in the control (Fig. 3a(iii)) indicating that the blue stimuli were effective in inducing the PDA effect during anoxia. Intracellular recordings show that the membrane was in a high conductance state (the PDA effect) when the cell first recovered from anoxia. This shows that the induction of PDA is just as effective during anoxia as in the normal state. c, We stimulated the eye with five blue pulses and then three orange pulses while the animal was in an anoxic state. There was no observable response to any of these stimuli. After recovery, the response to orange light showed that the eye was in the orange-adapted state (see Fig. 3a(iv)). Since the amounts of blue light and orange light given in the anoxic state were the same as those used in the control (Fig. 3a), the results indicate that the induction of PDA and the reversal of this process were just as effective during anoxia as in the normal state.

from anoxia. In Fig. 3c we show the responses from an animal which was in the state of anoxia when the blue stimuli and the three subsequent orange stimuli were applied. None of these stimuli induced any observable response. The next few orange stimuli were applied after the animal had recovered from anoxia. As in the case of the control experiment (Fig. 3a(iii) and (iv)) the responses to these orange stimuli show a full recovery from the PDA effect induced by the blue stimuli. The results shown in Fig. 3 indicate that the induction of PDA and its reversal by orange light are just as effective during anoxia as in the normal state, even though there is no observable photo-response during anoxia. Experiments similar to the above have also been carried out using lowered temperature (on *Drosophila*) or metabolic poisons (on *Balanus* by R. Clark Lantz, A.M. and F.W.) as blocking agents of photo-responses. Results from these experiments are consistent with those shown in Fig. 3.

Since the spectral transition of the pigments and the induction of the PDA effect all took place during anoxia when no response was observed, the observation that the conductance increase associated with the PDA appears after the animal has recovered from anoxia indicates that the shifting of rhodopsin to metarhodopsin does not increase the conductance directly. There must be intermediate steps "tightly coupled" to the shifting of the pigments, or to the pigment states themselves which lead to the change in membrane conductance. Our results show that one or more of the early steps initiated by the shifting of the pigments is not sensitive to anoxia. Once initiated, it persists through the period of anoxia and expresses its effect on recovery. One or more of the later steps in the chain of events leading to a conductance increase is, however, sensitive to anoxia. But our results do not rule out the possibility that the anoxia-insensitive steps could be the metarhodopsin state itself.

The above results have been obtained by studying the PDA phenomenon. Several lines of evidence suggest that the mechanism of PDA generation closely resembles that involved in the generation of the light-coincident receptor potential^{17,18}. Thus, the conclusions presented here may apply also to the light-coincident transduction process.

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Blood pressure in snakes from different habitats

SNAKES seem to have evolved from burrowing lizards and are experiencing an impressive adaptive radiation. Terrestrial forms represent the probable ancestral type which gave rise to several lines of divergent radiation into aquatic and arboreal habitats^{1,2}. These shifts in habitat may have affected blood pressure regulation in snakes which, because of their shape, are inordinately subject to the hydrostatic effects of gravity. By acquiring aquatic habits, some species have eliminated most of the hydrostatic stresses on the cardiovascular system; conversely, arboreal species are potentially exposed to serious problems of blood flow and perfusion pressure. Our study indicates that adaptive evolutionary changes in arterial blood pressure regulation occurred during the transitions from terrestrial to aquatic or arboreal life in snakes. There are distinct trends towards low arterial pressure, with reduced regulatory ability in aquatic species and towards high pressure, with good homeostasis in an arboreal species.

To examine blood pressure and the extent of its control, we measured arterial pressure during tilting experiments involving nine species from aquatic, semi-aquatic, terrestrial and arboreal habitats (Table 1). Freshly captured animals were anaesthetised in ice and the caudal segment of the aorta occlusively catheterised with saline-filled, heparinised, polyethylene tubing. The animals were straightened out in appropriately sized Perspex tubes and allowed to recover from the surgery for at least 20 h before pressure measurements were taken. The temperatures of the snakes were maintained between 26 and 29 °C. Blood pressure was measured with Bell and Howell or Statham transducers, calibrated with water manometers and coupled to a Beckman Dynograph or Grass Polygraph. The transducers were placed at heart level and the tilt angle, head-heart distance, and total length of the snake in the tube were measured for calculations of pressure in the head and body centre. After each change in tilt angle, there was a large immediate change in pressure followed by a period of adjustment after which the pressure stabilised. The negligible kinetic energy of blood in snakes is ignored.

There is good correlation between habitat and both the absolute arterial pressure and the response to tilting. In the aquatic and semi-aquatic species, the trend is towards lower pressure (Table 1) as well as a decreased ability to compensate for the effects of postural change (Fig. 1). The opposite trend appears in the arboreal species. Phyletic comparison is especially good between the terrestrial elapids (*Notechis scutatus* and *Austrelaps superbus*) and their close relatives, the sea snakes (*Laticauda colubrina*, *L. semifasciata*, *Hydrophis ornatus*, and *H. belcheri*)^{3,4}. The trends are shown independently by the arboreal snake, *Boiga dendrophila*, and the semi-aquatic snake, *Cerberus rhynchops*, which are both venomous rear-fanged colubrids. Only the non-venomous *Chersydrus granulatus* lacks a taxonomically close relative within the study. Like the other aquatic snakes, however, it shows relatively low blood pressure and

Table 1 Mean arterial blood pressure and its regulation in various snakes

Species (n)	Habitat	Mean b.p.* (mmHg)	Weight (g)	Total length (cm)	Head-Heart distance† (%)	Slope‡
<i>Boiga dendrophila</i> (3)	Arboreal	74	185	126	17	+0.25
<i>Austrelaps superbus</i> § (2)	Terrestrial	61	119	66	17	+0.01
<i>Notechis scutatus</i> (9)	Terrestrial	49	282	93	16	+0.14
<i>Cerberus rhynchops</i> (3)	Semi-aquatic	35	128	64	24	+0.05
<i>Laticauda colubrina</i> (3)	Semi-aquatic	38	495	116	30	+0.07
<i>L. semifasciata</i> (3)	Semi-aquatic	32	431	110	20	+0.02
<i>Chersydrus granulatus</i> ¶ (2)	Aquatic	27	239	77	43	-0.03
<i>Hydrophis belcheri</i> (2)	Aquatic	33	418	92	38	-0.23
<i>H. ornatus</i> (3)	Aquatic	22	512	97	34	-0.10

*Horizontal

†Head-heart distance \times 100/total length.

‡Slope of regression line fitted to head-up tilting data (Fig. 1).

§Formerly *Denisonia superba*.¶Formerly *Acrochordus granulatus*.

reduced control. The semi-aquatic and aquatic species represent three, or possibly four, independent shifts from land to water, each shift apparently accompanied by a consistent change in blood pressure regulation.

In Fig. 1 we show blood pressure at the centre of the snake, where there should be no pressure change because of tilting alone. If pressure remains constant at this point during tilting, then the snake is acting as a simple fluid-filled tube and is sustaining, in its upper half, a drop in pressure equal to the change in the hydrostatic blood column. Should the pressure at the centre of the snake increase with tilting, then there is physiological adjustment to augment pressure in the upper half; a fall in central pressure means that the pressure in the upper half falls excessively, probably because cardiac output is reduced by venous pooling in the lower half. Table 1 gives the slopes of regression lines fitted to the data for head-up tilting. *B. dendrophila* and *N. scutatus* are the best regulators and the two *Hydrophis* species are the poorest.

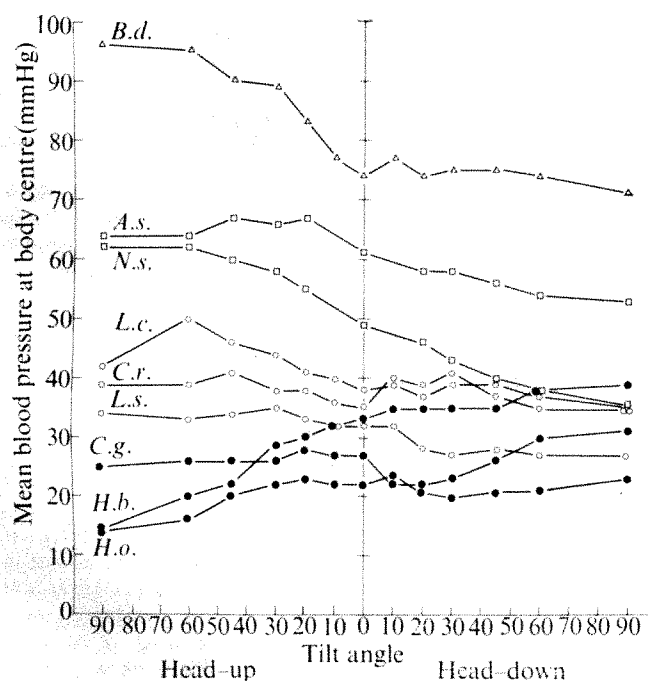


Fig. 1 Mean arterial blood pressure at the body centre in snakes as a function of tilt angle. Values are stable mean pressures following the initial adjustment (see text). Initials designate the species which are listed in Table 1. Symbols divide the species according to habitat: arboreal (Δ), terrestrial (\square), semi-aquatic (\circ), and aquatic (\bullet).

The physiological adjustments responsible for regulation of blood pressure are complex and are beyond the scope of this paper but our data indicate that regulation is achieved through changes in both heart rate and vasomotor tone (H.B.L. and R.S.S., unpublished). As in lizards⁵, vasomotor tone seems to be the most important factor in snakes.

The loss of regulatory control in aquatic snakes is probably attributable to the equalising effect of external hydrostatic pressure. Because totally aquatic snakes are never subjected to significant changes in transmural pressure, the need for regulation of venous tone does not exist. Alternatively, there should be strong selective advantage for vasomotor reflexes in snakes which move in three dimensions out of water. It is perplexing that, when measuring interstitial fluid pressure in large snakes during tilting, Scholander and coworkers⁶ found better homeostasis in the aquatic anaconda than in the arboreal boa constrictor. They did not measure blood pressure, however, and the pressure relationships in snakes between blood and interstitial fluid are unknown.

A possible adaptive value for higher absolute pressure in terrestrial and arboreal snakes is one of minimising the effects of postural changes on perfusion. As a simplified hypothetical example, assume that two similarly sized snakes having different blood pressures are tilted and both maintain constant central pressure. Because arterial pressure in the head is a function of vertical distance between the body centre and the head, both snakes experience the same absolute pressure decrease in the head, but the one with the higher central pressure shows a lesser proportionate change in head perfusion. It seems to be important for snakes to maintain adequate perfusion pressure in the brain, some snakes 'faint' if postural changes prevent sufficient blood flow to the head and reptiles generally do not tolerate stagnant anoxia (lack of oxygen resulting from stopped circulation) in the brain⁷. Blood pressure in the heads of the two *Hydrophis* species became negative at tilting angles of 45° or greater. At 90°, both *Laticauda* species and *C. granulatus* exhibited negligible head blood pressure.

In relation to the importance of maintaining pressure in the brain, it is interesting that the heart is proportionately closer to the head in terrestrial and arboreal snakes than in aquatic ones (Table 1). There is thus a trend in terrestrial snakes towards limiting the length of the vertical blood column to that which the heart can support. In aquatic species, on the other hand, the trend is towards a midbody position of the heart. This tends to equalise the flows and pressures required to perfuse the anterior and posterior portions of the animal.

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Effect of plant cytokinins on microfilaments and tight junction permeability

IN epithelia classified as "leaky", net transport efficiency of NaCl and water may be determined not only by cellular metabolic activity, but also by tight junction permeability and the conjugate physical driving forces acting between lumen and intercellular space. The significance of paracellular ion movement across leaky epithelia would become more apparent if a cytoplasmic mechanism for regulation of tight junctional permeability could be defined. The structure-function observations reported here suggest that the microfilament system may have such a function. Rapid and reversible increases in shunt path electrical resistance were observed in *Necturus* gallbladder exposed to plant cytokinins. In electron micrographs zeiotic transformation of the luminal membrane was associated with

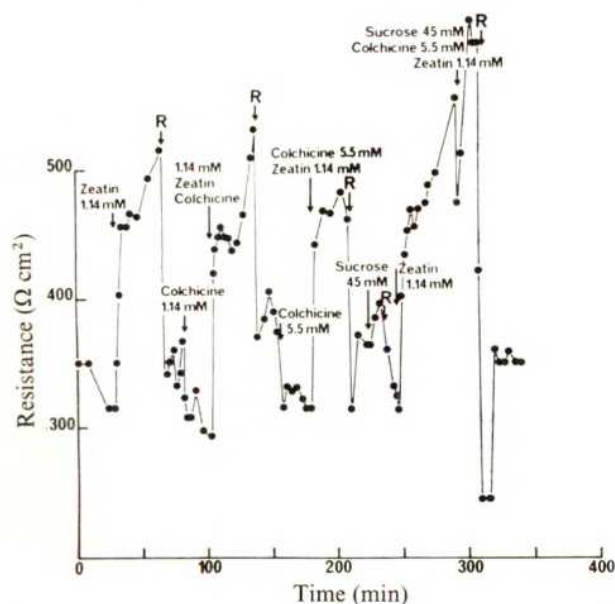


Fig. 1 An experiment illustrating resistance response to zeatin (1.14 mM in the mucosal bath). Transepithelial resistance is given on the ordinate. The initial increase in resistance occurs within 5 min and is completely reversible when zeatin is replaced by Ringer solution (R) containing the same concentration of DMSO. In this experiment, colchicine caused a decrease in resistance and did not inhibit the zeatin response.

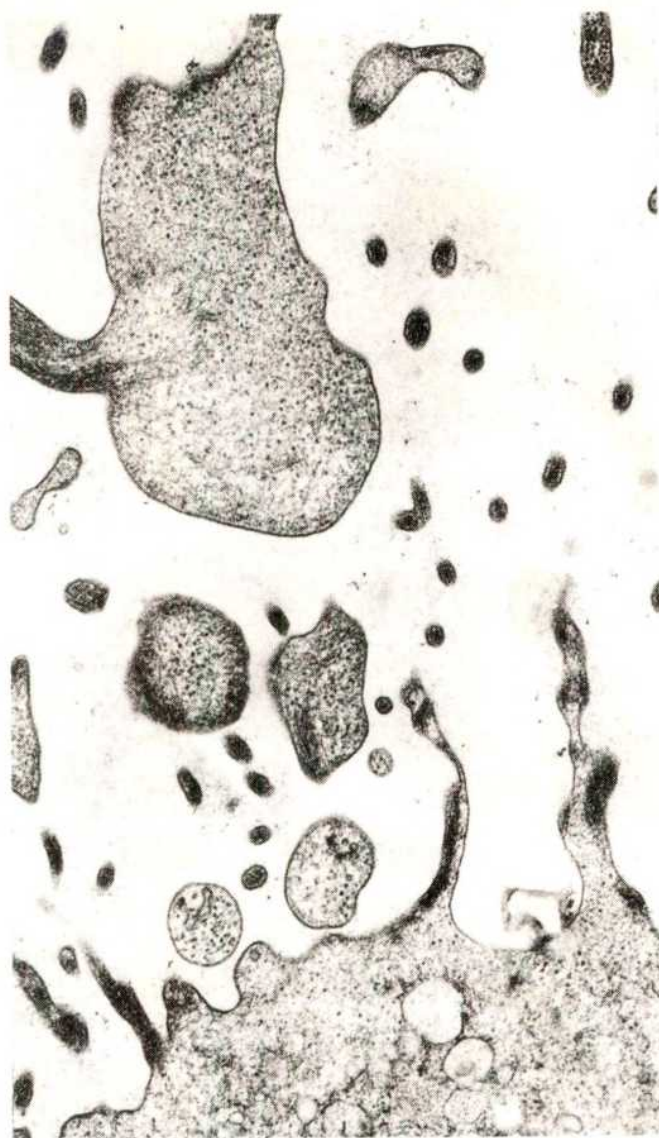


Fig. 2 Zeiosis following a 30-min mucosal exposure to kinetin (1.0 mM). Bizarre, enlarged microvilli demonstrate disrupted, clumped and swollen microfilaments. ($\times 24,500$).

disruption of microfilaments and in freeze fracture, with reorientation and disorder of tight junctional elements.

Gallbladders were slightly stretched over a 0.35 cm² opening in a Plexiglas chamber, mucosal side up¹; experiments were carried out at room temperature. The composition of the standard Ringer bathing solution was (in mM l⁻¹) NaCl 95, NaHCO₃ 13, CaCl₂ 1.8, MgCl₂ 1.0, KCl 4.5, KPO₄ 0.7, glucose 2.0 (220 mosmol l⁻¹), pH 7.4. Polyvinylpyrrolidone (molecular weight, 40,000, 20 g l⁻¹), was added to the serosal solution. Transepithelial potential difference (PD_{tr}) and resistance (R) were measured, the latter by a four-electrode technique. Relative cell membrane resistances were measured by the voltage divider ratio through an intracellular microelectrode.

Plant cytokinins are purine derivatives best known for their ability to induce plant cell division. Kinetin, zeatin, or zeatin riboside were dissolved in dimethyl sulphoxide (DMSO) and then in the Ringer's solution (final concentration of DMSO was no more than 0.5%). R and PD_{tr} were evaluated for mucosal cytokinin concentrations of 0.1–2.0 mM. Bladders were fixed *in situ* with 1 or 2% glutaraldehyde in 0.05 M phosphate buffer, pH 7.0. Freeze fracture was done at -150°C on 20% glycerol-impregnated tissue, previously fixed in glutaraldehyde.

In the control state, transepithelial electrical parameters were similar to those reported by others^{1–3}. PD_{tr} ranged from 0 to -6 mV, mean -1.74 mV ± 0.45 (s.e.) ($n = 12$), mucosal side

negative. The resistance averaged $236 \Omega \text{ cm}^{-2} \pm 26$ (s.e.) ($n = 11$). Dilution potentials, achieved by isosmotic substitution of 45 mM sucrose for NaCl, yielded a $9.9 \text{ mV} \pm 0.6$ (s.e.) ($n = 8$) shift in PD_{tr} , mucosa positive, indicating that the shunt pathway is cation selective. Cell PD_{tr} ranged from -39 to -66 mV , mean $-44.1 \text{ mV} \pm 0.7$ (s.e.) ($n = 27$).

Addition of cytokinins to the mucosal compartment did not change PD_{tr} , cellular PD or dilution potentials. Transepithelial resistance increased rapidly during the first 1–5 min reaching a peak value in 20–30 min and was rapidly reversible (Fig. 1). Cytokinin added to the serosal bath alone caused a delayed increase in resistance, but did not add to the mucosal response. The increase in resistance was roughly linearly dependent on mucosal concentration. Resistance doubled with mucosal bath concentrations of 2.0 mM zeatin or kinetin. The voltage divider ratio (R_{mucosa}/R_{serosa}) in four bladders decreased from 1.25 ± 0.13 to 0.79 ± 0.04 (s.e.) ($n = 23$) because of a decrease in R_{mucosa} . All changes were reversible after the cytokinin was washed from the mucosal compartment. Kinetin, the least soluble, gave the most gradual and persistent response. The apparent decrease in luminal membrane resistance induced by cytokinins may result from increased surface and/or a nonspecific distension of the luminal membrane, an interpretation suggested by the ultrastructure. Focal evagination of the luminal membrane (zeiosis)⁴ associated with disruption of attached microfilaments (Fig. 2) was observed as early as 5 min after cytokinin exposure, initially appearing at distal extensions of microvillae located at the apogee of the luminal membrane.

The concomitant increase in transepithelial resistance can safely be ascribed to an increase in shunt (tight junction plus intercellular space) resistance, previously reported to be 12–26 times smaller^{1,3} than R_{serosa} plus R_{mucosa} . More explicitly, current passage must be impeded at the tight junction, since no morphological changes were observed in the intercellular space and R_{mucosa} fell after cytokinin treatment.

In tissue exposed to cytokinins, desmosomes seemed more

Fig. 3 Freeze fracture (EF face) in control gallbladder exposed to Ringer with 0.5% DMSO. The furrows are regularly spaced and horizontally oriented. The most abluminal element (arrow) is continuous with rare basal extensions ($\times 59,100$).

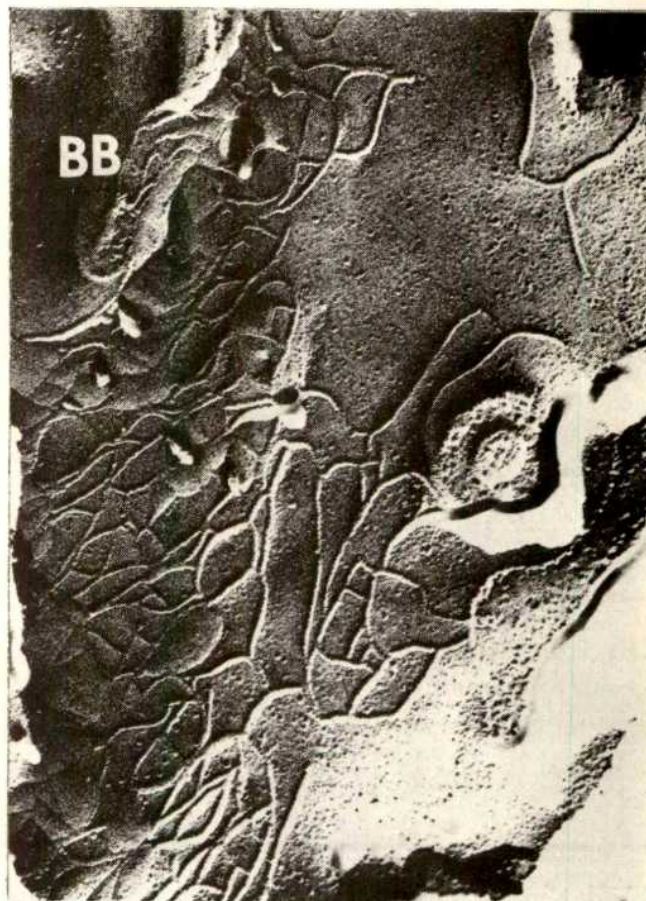
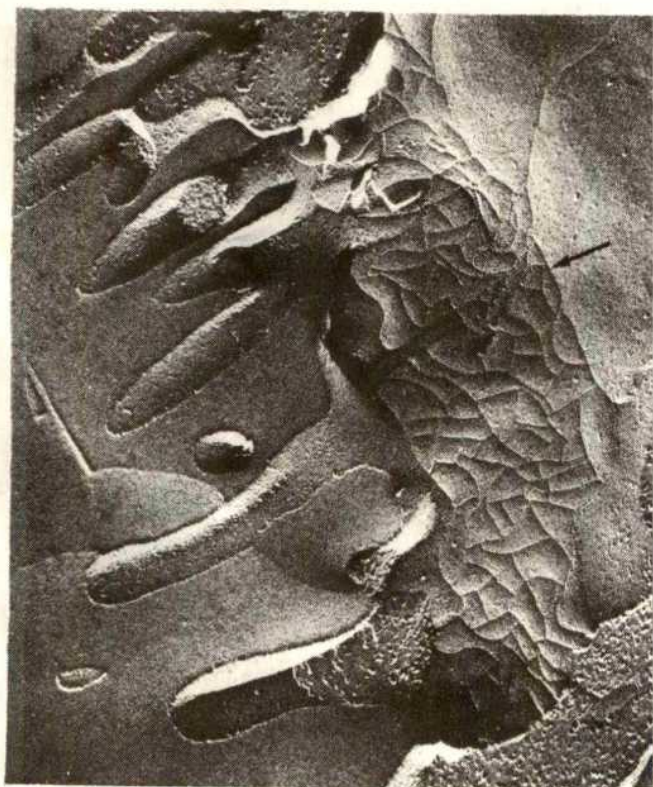


Fig. 4 Freeze fracture (PF face) 1 h after exposure to 1.0 mM zeatin. The brush border on the left is labelled (BB). Ridges are disorganised and oriented more perpendicular to the brush border than in control. The most abluminal ridge is discontinuous and open ends are often randomly directed down the lateral cell membrane ($\times 59,100$).

numerous and were frequently asymmetrical with short tonofibrils. With freeze fracturing, clusters of intramembranous particles on EF and PF fracture faces were found. All of these features are characteristic of desmosome assembly⁵. In electron-micrographs "fusion" lines between cells comprising the zona occludens (tight junction) showed focal areas of slight separation. The zona occludens seemed accentuated by clumping of microfilaments in the adjacent cytoplasm, whereas in control sections, most microfilaments were in apposition to the zona adherens and not the zona occludens.

On freeze fracture (Fig. 4), the furrows and ridges characteristic of tight junctions were not arranged in the regular configuration seen in control bladders (Fig. 3). Junctional strands seemed to change orientation from parallel to perpendicular to the luminal border and single open ended strands extended deep in the basal direction. The network was both more extensive and more disordered; in some areas, parallel ridges abutted and in other areas were widely separated. At basal extensions of the junction, new strand formation was suggested by beading and membrane indentation. These findings are similar to that reported in rat liver exposed to phalloidin, a drug also associated with ultrastructure changes in microfilaments⁶.

We postulate that control of paracellular route of ion permeation, dominated by tight junction, involves specific interaction between microfilaments and the specialised junctional domains of plasma membrane. Since the electrical and structural changes occur within a short period after mucosal exposure to cytokinins and are reversible, it is unlikely the new protein synthesis is required, and therefore, the observed alteration in

junctional reorganisation is due to re-use of pre-existing junctional components. The exact molecular mechanism, possible intermediary steps, and the modifying role of cytokinin action between the microfilament system and junctional assembly are not yet elucidated. These studies do, however, indicate that microfilaments are intimately associated with development and function of a type of plasma membrane specialisation, the tight junction (and possibly the desmosome). Most importantly, these data could lead to an understanding of the physiological control of tight junction permeability and its relationship to epithelial transport.

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Evidence for a dual role for oxygen in control of abscission

ABSCISSON—the shedding of leaves, fruits and other plant parts—is important in the morphogenesis and homeostasis of plants, and valuable agricultural practices are based on its control. Carns *et al.*¹ demonstrated that oxygen is essential for abscission; they found that less than 21% O₂ retarded abscission while more than 21% and up to 40% O₂ accelerated abscission; concentrations above 40% gave a uniform maximum rate of abscission (inset of Fig. 1). Acceleration of abscission by O₂ was confirmed in two investigations^{2,3}, but Abeles and Gahagan⁴, who studied abscission responses to O₂ in the presence of 0.01 p.p.m. ethylene, observed no acceleration with O₂ concentrations above 21%. They suggested that the accelerations observed before were caused by ethylene, which sometimes contaminates commercial O₂. We have re-examined the abscission responses to O₂ in carefully monitored conditions and found an unusual double sigmoid relationship between abscission and oxygen concentration.

Explants of cotton (excised cotyledonary nodes of *Gossypium hirsutum* L. cultivar Acala SJ-1) were held at 30 °C in small chambers with a continuous gas flow. O₂, CO₂ and ethylene were monitored by gas chromatography. Twenty explants (40 abscission zones) were placed in each chamber; percentage abscission was determined after 48 h and plotted in Fig. 1. The flow system permitted an average fluctuation of $\pm 2\%$ in O₂ concentration, and explants varied about $\pm 10\%$ in their rates of abscission. Consequently, both variations were taken into account in fitting the curve to the points (see legend).

Figure 1 shows that abscission was retarded below approximately 10% O₂. With 10–20% O₂, abscission rates equalled control (air) rates. Above about 25% O₂, abscission was accelerated strongly.

We attribute the retardation of abscission by concentrations of O₂ below 21% to restriction of O₂ to respiratory processes. Abscission depends on the synthesis of pectinases

and/or cellulases⁵, which in turn depend on ATP. Carns⁶ found that respiratory inhibitors also inhibited abscission. Abeles and Gahagan⁴ found that the rate of abscission paralleled the rates of respiration (as measured by oxygen uptake). We suggest that the acceleration of abscission by more than 21% O₂ is due to a second O₂-requiring process that promotes abscission. The double sigmoid shape of the curve indicates that two distinct oxidative processes are involved.

Measurements of ethylene and CO₂ evolution from the explants gave some insight into the nature of the acceleration of abscission by high concentrations of O₂. Ethylene production of the explants with 5–40% O₂ was essentially independent of O₂ concentration (ethylene was determined with a relative accuracy of about 2 p.p.b.). These data ruled out the possibility that the acceleration resulted from an increased production of ethylene due to the influence of O₂. The CO₂ output did not increase appreciably in high concentrations of O₂, suggesting that the acceleration of abscission was not due to general stimulation of respiratory processes. This is in accordance with the generally accepted view that O₂ does not limit respiration in concentrations above 21%, or even above the extinction point in some cases⁷. (Data for ethylene and CO₂ are being published elsewhere.) In a separate experiment abscission rate was measured in nine chambers receiving high concentrations of O₂ containing vials of mercuric perchlorate to absorb ethylene. There was strong acceleration of abscission in each chamber although ethylene never exceeded 3 p.p.b., well below the level that can influence abscission. Thus, contamination of O₂ by ethylene was not a significant factor in our experiments.

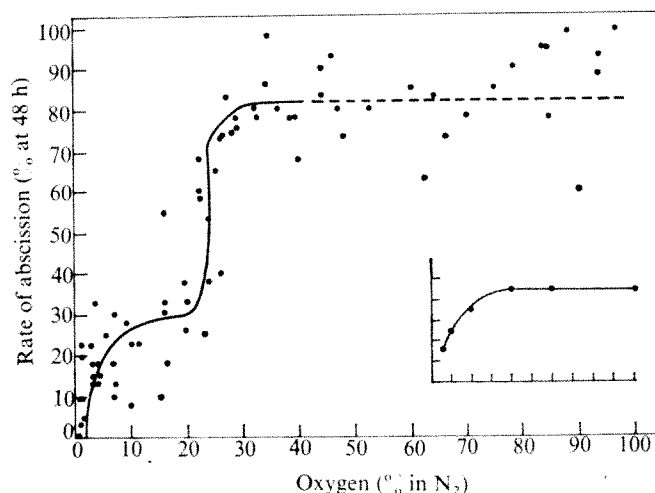


Fig. 1 Rate of abscission of cotton explants as influenced by oxygen concentration. There are 78 data points from experiments conducted in five different weeks with each point representing the percentage abscission of 40 abscission zones. Details of the operation of the flow system are given in ref. 9. Points from 0 to 40% O₂ were subjected to a detailed statistical analysis, taking into account errors in both abscission and O₂. Orthogonal regression¹⁰ on the function

$$f(x) = a + \frac{b}{(x+1)} + c \tanh[d(x-e)]$$

with unit weights and a constant scale factor of 5/7 applied to the y variable yielded the double sigmoid curve shown (solid line). The above function was chosen for its ability to assume either a smooth form or a double sigmoid form. Abscission is apparently independent of oxygen concentration in the 40–100% range (dashed line). The final variables and standard error of the best fit are: $a = 58.16$; $b = -80.20$; $c = 23.54$; $d = 10.01$; $e = 24.78$; $s.e. = 3.92$. Normalisation using air controls, and high O₂ concentration controls resulted in similar double sigmoid curves. (Details of the statistical analysis will be presented elsewhere.) The inset shows the effect of oxygen concentration (x axis) on the abscission rate (y axis) of bean explants held in sealed containers. Each point is an average of the response from 20 to 90 abscission zones (after Carns *et al.*¹).

There are several possible reasons why Abeles and Gahagan⁴ did not observe acceleration of abscission by high concentrations of O₂. First, the presence of 0.01 p.p.m. ethylene in their experiments would have impaired their ability to detect acceleration. Second, their measurements of abscission were made only 4 h after explants were exposed to the experimental atmospheres, and it is possible that high concentrations of O₂ are effective only after a longer time. Third, acceleration of abscission by high concentrations of O₂ may be due to interference with the insensitivity to ethylene that commonly follows explant excision. If the acceleration we report does represent a response to this ethylene, Abeles and Gahagan could not have observed it, for 19 h elapsed between explant excision and exposure to O₂ in their experiments. Finally, the ability of O₂ to accelerate abscission may vary from species to species and even from variety to variety.

Ethylene could still be indirectly related to this acceleration through an effect of O₂ on tissue sensitivity to ethylene, particularly during the first 12 h after excision. Another possible reason for the effect of high concentrations of O₂ is oxidative inactivation of indolyl acetic acid (auxin) catalysed by the enzyme complex known as "IAA-oxidase". Its proposed free radical mechanism⁵ could be responsive to high concentrations of O₂. Increased IAA-oxidase activity and decreased concentrations of auxin are known to be correlated with abscission⁶. It is also possible that some other process that requires a high concentration of O₂ accelerates abscission.

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Photosynthetic regulatory protein found in animal and bacterial cells

WE recently reported that two proteins, assimilation regulatory protein a (ARP_a) and assimilation regulatory protein b (ARP_b), are needed for the light-induced activation of certain enzymes of photosynthetic CO₂ assimilation^{1,2}. ARP_a and ARP_b link the strong reducing power of ferredoxin, reduced photochemically, to the control of key enzymes through a mechanism that, until now, was considered to be unique to chloroplasts^{3,4}. In a search to determine the distribution of this type of control mechanism, we have observed that the more thoroughly characterised of these proteins, ARP_b, occurs widely in living cells. The results provide evidence that protein modification reactions of the ferredoxin-linked type described for chloroplasts occur in non-photosynthetic as well as photosynthetic organisms.

ARP_b from different sources was assayed by its capacity to activate homogeneous preparations of spinach chloroplast fructose 1,6-bis-phosphatase^{1,5} in a system in which the chloroplast ferredoxin and ARP_a components were replaced by the non-physiological sulphydryl reagent dithiothreitol^{2,4,5}. In this assay, the inorganic phosphate cleaved from the C₁ position of fructose 1,6-bis-phosphate

Table 1 Occurrence of ARP_b in different types of photosynthetic organisms

	ARP _b (units per mg protein)
Green plants	
Spinach chloroplasts	3.3
Sugar beet chloroplasts	8.1
Blue-green algae	
<i>Nostoc muscorum</i>	0.6
Photosynthetic bacteria	
<i>Chlorobium thiosulfatophilum</i>	6.2
<i>Chromatium vinosum</i>	1.5
<i>Rhodospirillum rubrum</i>	1.1

The soluble protein fraction from isolated spinach chloroplasts (chloroplast extract) and cell-free extracts of the different micro-organisms were prepared in a solution containing 25 mM Tris, pH 8.0, and 1.4 mM 2-mercaptoethanol, as described previously^{3,4,6}, and adjusted to pH 4.5 with 2 N formic acid. The precipitate was centrifuged off (5 min, 48,200g); the supernatant fraction was adjusted to pH 7 with 1 N ammonium hydroxide and dialysed overnight at 4 °C against the preparative solution. For assaying ARP_b, the reaction mixture contained the indicated dialysed pH 4.5 supernatant fraction, fructose 1,6-bis-phosphatase, 8 µg, and the following (in µmol to a final volume of 0.5 ml): Tris-HCl buffer (pH 7.9), 50; MgCl₂, 0.5; dithiothreitol, 2.5; and fructose 1,6-bis-phosphate, 3. The reaction was started by adding fructose 1,6-bis-phosphate and was continued for 30 min at 25 °C. The reaction was stopped by adding 0.5 ml of 10% trichloroacetic acid and, after centrifuging off the precipitate, inorganic phosphate was estimated by a modified Fiske-SubbaRow procedure⁷. Protein was estimated by a modified phenol reagent procedure⁸.

by the phosphatase enzyme is measured colorimetrically. The assay gave reproducible results with ARP_b in cell-free preparations from which the native fructose 1,6-bis-phosphatase had been largely or totally removed by precipitation at pH 4.5 and dialysis of the resulting supernatant fraction overnight at alkaline pH (refs 4 and 5). One unit at ARP_b is defined as the ARP_b-mediated release of 1 µmol of inorganic phosphate by 8 µg of fructose 1,6-bis-phosphatase enzyme in the conditions in Table 1.

Table 1 shows that ARP_b was present in comparable amounts in dialysed pH 4.5 supernatant fractions obtained from the major types of photosynthetic organisms: higher plants (chloroplasts of spinach and sugar beet), algae (*Nostoc muscorum*), and each of the three major types of photosynthetic bacteria (green sulphur, *Chlorobium thiosulfatophilum*; purple sulphur, *Chromatium vinosum*, and purple non-sulphur, *Rhodospirillum rubrum*). Such a distribution of ARP_b in photosynthetic cells prompted us to examine non-photosynthetic counterparts for this protein. This search led to the finding of ARP_b not only in non-photosynthetic plant tissues (spinach seeds and roots) but also in heterotrophic bacteria—organisms which obtain energy by the breakdown of organic compounds and which cannot utilise radiant energy (Table 2). ARP_b was found in heterotrophic bacteria of both the aerobic (*Escherichia*

Table 2 Demonstration of ARP_b in non-photosynthetic plant tissues and heterotrophic bacteria

	ARP _b (units per mg protein)
Plant tissues	
Spinach seeds	3.1
Spinach roots	1.6
Aerobic bacteria	
<i>Escherichia coli</i>	4.9
<i>Bacillus subtilis</i>	1.6
Anaerobic bacteria	
<i>Clostridium pasteurianum</i>	1.3
<i>Desulfovibrio desulfuricans</i>	2.0

Cell-free extracts of seeds and roots were prepared by blending tissues and centrifuging the resulting slurry as in Table 1. The ratio of tissue to buffer for blending was 1:2 (weight:volume). Before disruption, seeds were soaked for 8 h in flowing tap water. Other conditions were as in Table 1.

Table 3 Effect of light on the development of ARP_b in barley seedlings

	ARP _b (units per mg protein)
Seedlings germinated in darkness	9.6
Seedlings germinated in light	7.5

Barley seedlings were germinated from seeds placed in vermiculite saturated with water. The pH 4.5 supernatant fraction was prepared from seedlings germinated for 7 d at 25 °C and assayed as in Table 2.

coli, *Bacillus subtilis*) and anaerobic (*Clostridium pasteurianum*, *Desulfovibrio desulfuricans*) types. Additional evidence that the formation of ARP_b is independent of light came from the finding that light- and dark-germinated barley seedlings contained similar amounts of ARP_b (Table 3).

The occurrence of ARP_b in non-photosynthetic plant and bacterial cells raised the possibility that this protein might also be present in tissues of animal origin. Analyses were therefore conducted with dialysed pH 4.5 supernatant fractions from rabbit liver—a source rich in enzymes and

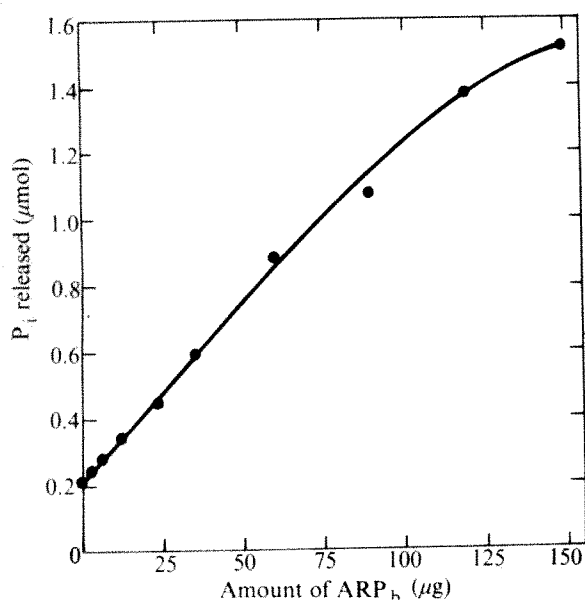


Fig. 1 The indicated amounts of a highly purified liver ARP_b preparation were assayed as described in Table 1. P_i corresponds to inorganic phosphate.

associated regulatory proteins. The initial results, while indicating the presence of ARP_b, revealed that in this case the preparation required additional purification to eliminate interference in the assay by the native fructose 1,6-bisphosphatase. Accordingly, the pH 4.5 supernatant fraction of liver cells was further fractionated by a procedure consisting of precipitation with ammonium sulphate and successive chromatography on Sephadex G-100, diethylaminoethyl (DEAE) cellulose and hydroxyapatite columns. The purified preparation so obtained consistently showed ARP_b activity (Fig. 1). Other properties of the purified liver ARP_b preparation will be published elsewhere.

In summary, the present findings show that ARP_b, a protein first described for chloroplasts, is widely distributed in nature. ARP_b was found in photosynthetic and non-photosynthetic plant cells, photosynthetic bacteria, anaerobic and aerobic heterotrophic bacteria, and animal cells. This distribution of ARP_b extends to the major types of living cells the possibility of a mechanism of protein modification of the reductive type that in chloroplasts is mediated by reduced ferredoxin. Those cells which lack a ferredoxin of the plant or bacterial type^{7,8} accordingly would utilise other reductants to this end. The identity of

the reductant used by such cells in lieu of ferredoxin is unknown.

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Native collagen is not a substrate for the collagen glucosyltransferase of platelets

THE adhesion of platelets to collagen in the subendothelial tissue is generally considered to be the primary step in haemostasis following vascular injury. The biochemical basis of this interaction is not fully understood but it has been suggested by Jamieson and coworkers^{1,2} that the adhesion of platelets to collagen is mediated by the formation of an enzyme-acceptor complex between a glucosyltransferase on the platelet membrane and incomplete carbohydrate residues present in collagen. We report here studies confirming the presence of collagen glucosyltransferase activity associated with platelets but investigations of the influence of collagen substrate conformation on enzyme activity suggest that the above hypothesis is probably untenable.

The enzyme assays used to study collagen glucosyltransferase activities associated with human platelets^{3–5} were originally used in studies claiming to show collagen glucosyl- and galactosyltransferase activities in guinea pig skin^{6,7}. These procedures have been criticised for their lack of specificity^{8,9} and methods designed to measure specifically the synthesis of hydroxylysine-¹⁴C-glycosides have subsequently been described^{8–11}. In this study, a preliminary characterisation of the collagen glucosyltransferase of porcine platelets has been carried out using the assay procedures of Harwood *et al.*¹¹. Platelets were prepared by the method of Lopaciuk and Solum¹² from blood anticoagulated with 5.4 mM EDTA. The preparations were sonicated

Table 1 Comparison of bivalent cations as cofactors for collagen glucosyltransferase

Cation added	Concentration (mM)	Glucosyltransferase activity (d.p.m.)	relative activity (%)
None		2,825	3
Mn ²⁺	10	91,850	100
	20	88,510	96
Mg ²⁺	10	31,218	34
	20	26,006	28
Co ²⁺	10	14,437	16
	20	15,240	17
Ca ²⁺	10	9,721	11
	20	8,196	9

The reaction using glucose-free corneal gelatin as substrate was carried out as described in Fig. 1 except that the metal cofactor was varied.

(2×5 s) at 4 °C in 0.02 M Tris-HCl buffer, pH 7.4, containing 0.15 M NaCl and 10 μ M dithiothreitol, dialysed against this buffer (200 volumes) and then assayed for collagen glucosyltransferase activity. The substrate employed was glucose-free bovine corneal gelatin which contained 11 hydroxylysine residues per 1,000 amino acids of which approximately seven residues were substituted with galactose. A linear relationship between enzyme concentration and the synthesis of 14 C-glucosylgalactosyl-hydroxylysine was obtained. Varying the substrate concentration gave Michaelis-Menten kinetics for the reaction and the K_m for glucose-free corneal gelatin was 5.35 g l $^{-1}$ (Fig. 1). Studies on the effect of pH on enzyme activity showed an optimum between pH 6.8 and 7.4, which differs markedly from the pH optimum of 5.7 initially determined for this enzyme in human platelets using a collagenous substrate³.

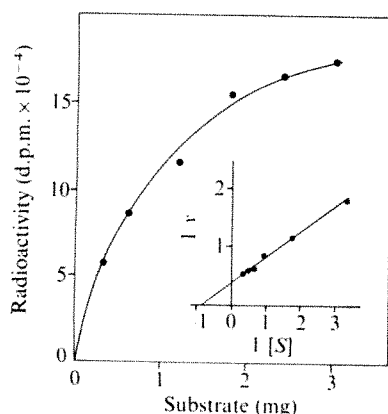


Fig. 1 Effect of the concentration of corneal gelatin on the glucosyltransferase activity of porcine platelets. Collagen was prepared and purified from bovine corneal tissue using pepsin solubilisation and salt precipitation techniques and then treated with 0.025 M H₂SO₄ for 20 h at 100 °C to remove selectively glucose residues. After hydrolysis the sample was neutralised with 2.0 M NaOH, and varying amounts of this substrate were used directly in the assay. The incubation was carried out for 90 min at 37 °C in a total volume of 200 μ l which also contained 15 μ mol of Tris-HCl buffer, pH 7.4, 2 μ mol MnCl₂, 0.4 μ mol of 2-mercaptoethanol, 10 μ l of 0.1% Triton X-100, 0.67 μ Ci of UDP-C¹⁴ glucose, and 0.8 mg of platelet enzyme protein. Precipitation of the protein with acetone and subsequent determination of the 14 C-glucosylgalactosyl-hydroxylysine synthesised was achieved by alkaline hydrolysis, desalting and paper electrophoresis of the samples as described in ref. 11. In the double-reciprocal plot the values shown in the abscissa are reciprocal values of substrate concentration in mg per 200 μ l, and the values shown in the ordinate are reciprocal values of the velocity in 10 $^{-6}$ × radioactivity of the product formed in 90 min.

The effect of varied Mn²⁺ concentration on glucosyltransferase activity was examined and the optimum Mn²⁺ concentration found to be 10 mM. Experiments to determine whether other bivalent metals could replace the manganese indicated that Mg²⁺, Ca²⁺ and Co²⁺ were much less effective cofactors (Table 1). These results are similar to those reported for collagen glucosyltransferase from human platelets³, rat renal cortex⁸ and embryonic chick tissues^{10,12}. A further similarity to the human platelet³ and chick embryo enzyme¹³ is the susceptibility of the porcine platelet enzyme to marked inhibition by *p*-mercuribenzoate and *N*-ethyl maleimide: 50% inhibition occurred with both reagents at about 0.3 mM. These observations and the finding that the enzyme activity was also stimulated approximately 30% above controls by 2×10 $^{-4}$ M 2-mercaptoethanol suggest that free sulphhydryl groups are present in the active centre of the enzyme. Since platelet adhesion to collagen is inhibited by sulphhydryl inhibitors¹⁴, the above data can be

quoted in support of the collagen glucosyltransferase theory of collagen-platelet adhesion.

According to the hypothesis, however, the first step must be the interaction of the platelet enzyme with galactosyl-hydroxylysine residues in collagen. The use of hydroxylysine-galactose as substrate for the human platelet enzyme has been reported briefly¹⁵ but observations with this substrate cannot necessarily be extrapolated to support the hypothesis that the natural substrate of the platelet enzyme is native collagen. We have therefore re-examined this question and the effect of substrate conformation on the glucosylation reaction promoted by the platelet enzyme was studied by comparing the efficiency of native corneal collagen and heat-denatured corneal collagen as substrates. Because the collagen triple helix is readily denatured above 38 °C, these experiments were conducted at 30 °C to ensure that the native corneal collagen retained its triple helical conformation. A marked difference was observed between the native and denatured collagen in that when the reaction was studied as a function of time and enzyme concentration (Fig. 2), no synthesis of 14 C-glucosylgalactosyl-hydroxylysine was observed with native collagen. These results are analogous to those reported for the collagen glucosyltransferase involved in the intracellular glucosylation of procollagen¹⁶ and suggest that the platelet enzyme also requires the collagen substrate to be non-helical.

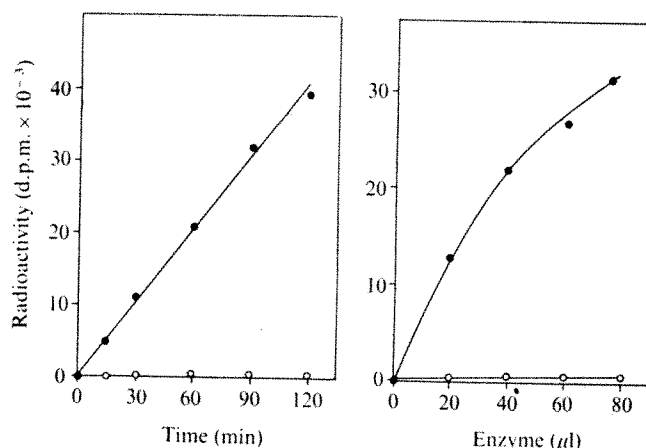


Fig. 2 Comparison of native and heat-denatured corneal collagen as substrates for collagen glucosyltransferase. The reaction was studied as a function of time and enzyme concentration and in both experiments the substrate concentration was 2 mg ml $^{-1}$. The native collagen used was prepared by pepsin digestion and purified under non-denaturing conditions. The optical rotation (α)_D of this collagen was found to be -430° which indicated that the collagen was in the triple helical form. The heat-denatured collagen substrate was prepared immediately before use by placing a portion of the above preparation at 60 °C for 10 min followed by rapid cooling in ice. The optical rotation of this denatured substrate was -135°. The reaction conditions were as described in Fig. 1, except that the incubation temperature was 30 °C. ○, Native (triple helical) substrate; ●, heat-denatured substrate.

Thus it seems that following vascular injury the adhesion of platelets to native collagen is unlikely to be mediated by the collagen glucosyltransferase. The roles of this enzyme and also collagen galactosyltransferase which we find to be present in porcine platelet preparations remain to be established.

Basic to the original hypothesis of Jamieson *et al.*^{1,2} were their observations that collagen glucosyltransferase activity was located primarily on the outer surface of the plasma membrane of human platelets³. We have undertaken a study of the location of collagen glucosyltransferase in subcellular fractions of porcine platelets isolated by the method

of Barber and Jamieson¹⁷. Using the specific enzyme assay we find that considerable activity occurs in the cytosol fraction as well as in the membrane and of particular interest is the presence of high amounts of collagen glucosyltransferase activity in freshly prepared platelet-free plasma; further details will be published later. The characterisation of the plasma enzyme and its relationship to the platelet enzyme are under investigation, but these observations must cast further doubts on the role of collagen glucosyltransferase in platelet-collagen adhesion.

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Changes in viral envelope structure preceding infection

THE first stage of infection by enveloped paramyxoviruses probably involves fusion of the viral envelope with the cell plasma membrane¹. During the course of an *in vitro* investigation into the mechanism of cell-cell fusion induced by Sendai virus it has become apparent that prior to the membrane fusion event the viral envelope undergoes a dramatic change in structure and this altered structure allows specific sites of fusion of viral envelopes with the plasma membrane to be identified in freeze fracture replicas.

Previous thin section studies have shown fusion of Sendai virus particles with the plasma membrane^{2,3} but these studies have not revealed any membrane specialisations which might occur. Freeze-fracture studies have shown a virus-induced aggregation of intramembranous particles during cell-cell fusion⁴ but have not shown actual fusion of viral envelopes with the plasma membrane.

In vitro, cell-cell fusion is carried out by first agglutinating a suspension of cells with Sendai virus at 4 °C. Fusion is a temperature-sensitive event, and occurs following a brief incubation at 37 °C (ref. 5). In these conditions fusion of viral envelopes with the cell membrane also occurs. In fact, cell-cell fusion probably requires an intermediate step involving fusion with the viral envelope⁶. In the present studies 1 ml of a 2% suspension of washed human erythrocytes (Type O) in Hank's Balanced Salt Solution⁷ (Hank's BSS) was agglutinated with 1 ml of

Sendai virus (1,000 haemagglutinating units ml⁻¹ for 1 h at 4 °C). Samples were then incubated for up to 1 h at 37 °C. Fusion was stopped by the addition of an equal volume of 6% glutaraldehyde and fixation continued for 30 min. Following infiltration with 25% glycerol cells were rapidly frozen in Freon 22 and fractured at -110 °C in a Denton freeze-fracture machine. Platinum carbon replicas were prepared for electron microscopy.

Freeze-fractured Sendai virus particles reveal internal aspects of their envelope structure. Fractures produce two complementary split membrane faces: P faces which belong to the cytoplasmic half of the membrane and face the exterior and E faces which belong to the external half of the membrane and face the cytoplasm⁸. At 4 °C, concave E fracture faces have numerous ~14-nm diameter intramembranous particles (Fig. 1a) while convex P faces have a complementary arrangement of pits (Fig. 1b). Following a brief incubation at 37 °C some virus particles undergo a dramatic change in the structural organisation of their envelopes. Virus particles are no longer spherical but develop a convoluted profile and such virus particles are characterised in freeze-fracture replicas by the presence of ~30-nm wide and up to 0.5-μm long smooth linear ridges on E faces (Fig. 1c) and by a complementary arrangement of linear grooves on P faces (Fig. 1d). Instead of ~14-nm diameter intramembranous particles on E faces

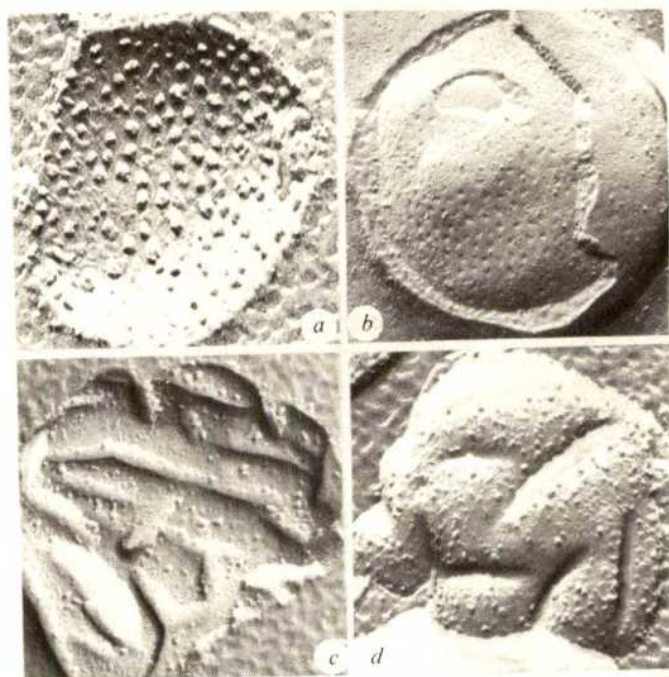


Fig. 1 Freeze-fracture replicas showing fractured Sendai virus particles at 4 °C (a, b) and at 37 °C (c, d). At 4 °C concave E fracture faces (a) display numerous ~14-nm diameter intramembranous particles and P faces a complementary arrangement of pits (b). At 37 °C concave E faces (c) of many virus particles now feature several ~30-nm wide smooth linear ridges and convex P faces (d) a complementary arrangement of linear grooves. ~9-nm diameter intramembranous particles are seen on the non-ridge regions of both P and E faces (c, d). *a* × 93,500; *b* × 51,000; *c* × 72,250; *d* × 82,875.

~9-nm diameter intramembranous particles can be seen on both P and E faces (Fig. 1c, 1d). These changes in the structural organisation of the viral envelope seem to be cell mediated and only take place when virus particles are bound to erythrocytes; free virus particles incubated and glutaraldehyde fixed at 37 °C remain spherical and display the same morphology as virus particles at 4 °C as in Fig. 1a and b).

Only virus particles having this altered morphology appear to be capable of fusing with the erythrocyte membrane; other virus particles which remain spherical never fuse and even after a prolonged incubation at 37 °C such spherical virus particles remain bound but unfused with the plasma membrane.

The smooth ridged regions of the viral envelope seem to be the initial site of fusion of the viral envelope with the erythrocyte membrane and the presence of these ridges allows specific sites of viral fusion to be recognised. Figure 2 shows a viral envelope which has fused and become completely incorporated into the erythrocyte membrane. In the conditions used there is no change in the distribution of erythrocyte intramembranous particles on either P or E fracture faces and at all times during agglutination and fusion intramembranous particles are randomly distributed.

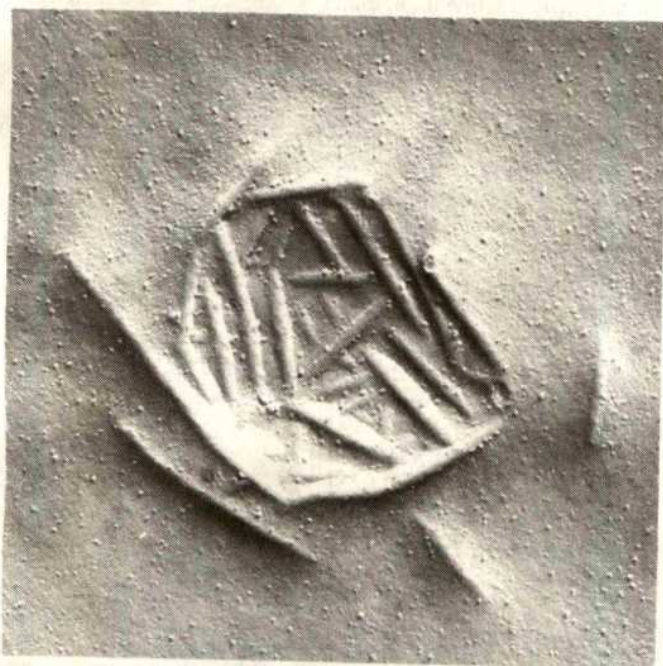


Fig. 2 Freeze-fracture replica showing the envelope of an unusually large virus particle which has fused and become completely incorporated into the E fracture face of an erythrocyte membrane ($\times 57,375$).

In summary, changes in the structure of the viral envelopes prior to fusion involve the loss of the ~14-nm diameter intramembranous particles from E faces, the appearance of ~9-nm diameter intramembranous particles on both P and E faces and a reorganisation of membrane components to produce the particle-denuded linear E face ridges. The biochemical changes which accompany these structural modifications are unknown.

Membrane fusion probably occurs when lipid bilayers in two opposing membranes are brought close enough to interact⁹. The particle-denuded linear ridge regions of the viral envelope probably represent such areas of lipid bilayer¹⁰. The observed membrane changes, therefore, are probably a specialised viral mechanism which allows fusion to take place as a result of interaction of the smooth ridge regions with the erythrocyte membrane. Although no gross changes in the distribution of erythrocyte intramembranous particles occurs, specific localised changes at the actual sites of fusion resulting in particle-denuded regions of erythrocyte membrane may occur possibly as a result of the viral neuraminidase.

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Cyclic AMP-dependent protein kinases from normal and SV40-transformed 3T3 cells

THE apparent molecular mechanism by which cyclic AMP regulates eukaryotic physiology is through the activation of cyclic AMP-dependent protein kinase enzymes¹. Two cytosolic protein kinase isozymes, called types I and II², have been described in mammalian tissues. They differ in their charge², dissociability by salt², capacity to undergo an autophosphorylation reaction³ and in their antigenic properties⁴. Evidence suggests that cyclic AMP regulates cell division. Intracellular levels of cyclic AMP are higher in normal than in transformed cells^{5,6}, and exogenous addition of cyclic AMP analogues to the culture medium inhibits the growth of transformed cells⁷. Cyclic AMP levels⁸, protein kinase specific activity⁹, and the relative amounts of types I and II isozymes¹⁰ vary during the cell cycle. A genetic lesion in lymphoma cells making them insensitive to cyclic AMP may be due to a defective protein kinase enzyme¹¹. We report here that cytosol from SV40-transformed 3T3 cells contains a type I protein kinase not found in BALB 3T3 cytosol. Chromatographic separation of these enzymes indicates that this isozymic difference is the result of an additional protein kinase regulatory subunit in SV3T3 cytosol.

Cell lines designated BALB 3T3 and SV3T3 originated from the BALB A₃₁ and SVT₂ lines, respectively (established by Dr George Todaro (NIH)); the Swiss 3T3 cell line was obtained from Dr Howard Green (MIT). The binding of cyclic AMP to protein was modified from the method of Gilman¹²; protein kinase assay was modified from Corbin and Reimann¹³; protein concentrations were estimated by the method of Lowry *et al.*¹⁴; and DEAE-cellulose chromatography of cytosol was modified from the method of Corbin *et al.*².

Initial studies indicated that the cyclic AMP-binding and protein kinase specific activities of cytosol fractions prepared from SV3T3 and 3T3 cells were similar, but not identical. The cyclic AMP binding activity (pmol ³H-cyclic AMP bound per mg protein) was 4.6 ± 1.1 for SV3T3 cytosol and 6.6 ± 2.7 for 3T3. The cyclic AMP dissociation constants (*K_d*), estimated from Scatchard¹⁵ analysis, were 2.3 ± 0.3 nM for SV3T3 and 3.3 ± 0.7 nM for 3T3. Cyclic AMP stimulated the SV3T3 protein kinase activity (pmol ³²P incorporated per mg protein per min) from 110 ± 30 to 710 ± 200, and the 3T3 kinase activity from 170 ± 40 to 1,100 ± 300. These activity ratios (—cyclic AMP/+ cyclic AMP) are approximately equal. Preincubation of crude cytosols at 60 °C before assay for cyclic AMP binding activity, however, indicated that the activity in 3T3 cytosol was more sensitive to heat denaturation than was the SV3T3 cytosol (Fig. 1). After 15 min at 60 °C, cyclic AMP-binding activity was

negligible in 3T3 cytosol, whereas SV3T3 cytosol still retained 25% of the activity. SV3T3 and 3T3 thus seemed to contain the same amount of cyclic AMP-dependent protein kinase activity, but these heat denaturation experiments suggested that the molecular structures of the protein kinase regulatory subunits were different. Chromatographic separation of these enzyme activities were carried out in order to examine this difference.

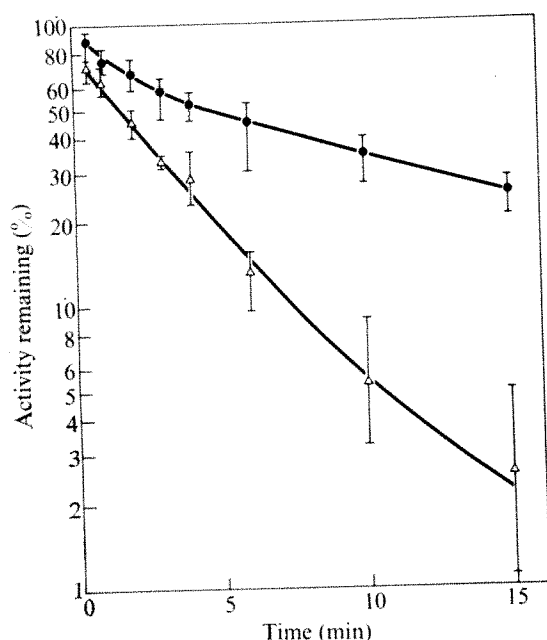


Fig. 1 The thermal stability at 60 °C of ^3H -cyclic AMP binding activity in SV3T3 (●) and BALB 3T3 (△) cytosols. Cells were grown in disposable glass roller bottles (Belco) using Dulbecco's modified Eagle's medium supplemented with 10% calf serum. Approximately 2×10^6 cells were seeded into the bottles with 100 ml of medium, and 5% CO_2 was added; the bottles (three for SV3T3 and 12 for 3T3) were capped and placed in a roller bottle incubator (Forma) for 4 to 10 d (until confluency). The medium was then removed and the cells were washed twice with 20 ml of cold 0.32 M sucrose. The cells were scraped into 5 ml of sucrose and collected by centrifugation at 150g for 10 min. Cells were disrupted by sonication and subjected to the following consecutive centrifugations: 3,000g, 10 min; 27,000g, 15 min; and 100,000g, 40 min. The final supernatant (cytosol) was used in all experiments. Tightly capped tubes containing cytosols were incubated at 60 °C for varying amounts of time, cooled, and the binding activity was measured. The 200- μl binding assay reaction volume contained 50 mM sodium acetate (pH 4.0), 20 nM ^3H -cyclic AMP (16 Ci mmol $^{-1}$, Schwartz-Mann) and 60–120 μg of cytosol protein. After 5 h at 0 °C the binding component-cyclic AMP complex was separated from unbound cyclic AMP by Millipore filtration. Filters were counted as described by MacKenzie and Stellwagen¹⁶. Boiled blanks were used for assays of crude cytosol. The data for the SV3T3 cytosol are the average of six experiments, those for BALB 3T3 the average of three experiments. Range bars are shown.

Figure 2 shows the protein kinase and cyclic AMP-binding elution profiles obtained following DEAE-cellulose chromatography of BALB and Swiss 3T3 cytosols, and from two different SV3T3 clones. Both SV3T3 lines showed two peaks (Peaks 1 and 2) of cyclic AMP-dependent protein kinase activity which were coincident with peaks of cyclic AMP-binding activity. Peak 1 eluted at roughly 0.05 M NaCl and Peak 2 eluted at roughly 0.18 M NaCl. The activity ratio of peak 1 was increased by the addition of NaCl, while that of peak 2 was unchanged (data not shown). These properties suggest that peaks 1 and 2 are similar to the types I and II protein kinases found by Corbin *et al.*² in mammalian tissue cytosol. Both BALB and Swiss 3T3 lines lacked a peak 1 protein kinase but did contain a peak 2 protein kinase. The elution profiles of cytosols from logarithmically growing and from confluent

(quiescent) BALB 3T3 cells were indistinguishable (data not shown).

When SV3T3 cells were treated for 20 h with medium containing 10^{-3} M N^6, O^2' -dibutyryl cyclic AMP (db cyclic AMP) and 0.5 mM aminophylline, the chromatographic pattern was considerably altered (Fig. 3a). Peak 1 contained no cyclic AMP-binding activity and had an activity ratio near unity, indicating that this protein kinase was independent of cyclic AMP. An additional peak containing 35% of the total cyclic AMP-binding activity and no protein kinase activity eluted after peak 1 and before peak 2. The amount of cyclic AMP-dependent protein kinase activity in peak 2 had decreased. When cytosol prepared from SV3T3 cells was treated with 10^{-4} M db cyclic AMP for 1 h (Fig. 3b), a similar elution profile to that found after the db cyclic AMP treatment of intact cells was obtained. When intact SV3T3 cells were washed just before collection with db cyclic AMP-containing medium, the elution profile was the same as that of cytosol from untreated cells. Thus, the db cyclic AMP treatment of intact SV3T3 cells was not a consequence of residual db cyclic AMP from the medium interacting with cytosols during sonication. The formation of a

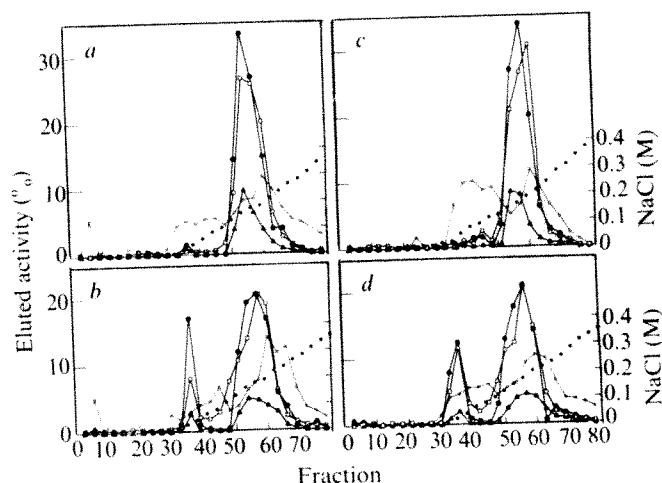


Fig. 2 DEAE-cellulose elution profiles of a, BALB 3T3; b, SV3T3; c, Swiss 3T3; and d, SV3T3_R cells. For a comparison of different samples, two identical columns were eluted from the same gradient mixer. DE-52 cellulose (Whatman) columns (0.9 × 10 cm) were equilibrated with 5 mM N -2-hydroxyethyl-piperazine- N' -2-ethanesulphonate (HEPES, pH 7.5) + 1 mM EDTA (buffer). After the application of 8–12 mg of cytosol protein, each column was washed with 30 ml of the buffer, and then eluted with a 60-ml linear salt gradient (0–0.4 M NaCl). The flow rates of each column were 10 ml h $^{-1}$; 75 fractions containing 1.2 ml were collected from each column. Conductivity measurements were used to determine the NaCl concentrations in the elution gradient. The 150- μl protein kinase reaction mixture contained 6.7 mM MgCl_2 , 20 mM 2-(N -morpholino)-ethane sulphonate (MES, pH 6.5), 300 μg histone (Type IIA, Sigma), 0.2 mM (γ - ^{32}P)ATP (about 20 c.p.m. pmol $^{-1}$), and 75 μl from a column fraction, with or without 10^{-6} M cyclic AMP. After incubating for 10 min (cyclic AMP present) or 20 min (cyclic AMP absent) at 30 °C, 100 μl of the reaction mixture was transferred onto filter paper squares (Whatman) and washed. γ - ^{32}P -ATP was prepared by the method of Post and Sen¹⁷. The Lowry *et al.* procedure¹⁴ was modified to include HEPES buffer in the bovine serum albumin references when protein concentrations of column fractions were estimated. Activities shown are ^3H -cyclic AMP binding activity (□), protein kinase activity in the absence (▲) and presence (●) of 10^{-6} M cyclic AMP, NaCl concentration (---), and protein (○).

cyclic AMP-independent protein kinase peak and a new peak of cyclic AMP-binding activity suggests that db cyclic AMP treatment of both intact cells and cytosol caused a dissociation of protein kinase holoenzyme into constituent catalytic and regulatory components. Treatment of cytosol from 3T3 cells with either cyclic AMP (Fig. 3c) or db cyclic AMP (Fig. 3d)

resulted in an elution profile different from that of untreated 3T3 cytosol and also distinct from the SV3T3 profiles. There was a decrease in the absolute amount of cyclic AMP-dependent protein kinase activity eluting at the peak 2 position, and the appearance of cyclic AMP-independent protein kinase eluting at the peak 1 position. The cyclic AMP-binding activity remained at the peak 2 position, and no peak of cyclic AMP binding appeared intermediate to peaks 1 and 2.

When the cyclic AMP-binding affinities and the cyclic AMP-dependent protein kinase specific activities of crude cytosol fractions from normal and transformed 3T3 cells were compared, no major differences were observed. This agrees with a previous study of protein kinase activities in normal and feline sarcoma virus-transformed cells¹⁸. Nevertheless, experiments testing the thermolability of the cyclic AMP-binding proteins in normal

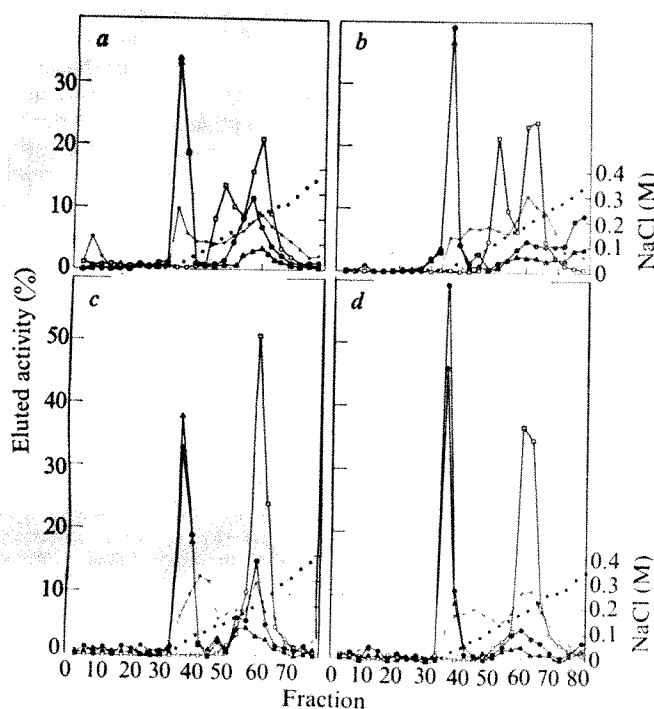


Fig. 3 DEAE-cellulose elution profiles of *a*, cytosol from SV3T3 cells treated with media containing 10^{-3} M db cyclic AMP + 0.5 mM aminophylline for 20 h; *b*, SV3T3 cytosol incubated with 10^{-4} M db cyclic AMP for 1 h; *c*, cytosol from BALB 3T3 cells incubated with 10^{-7} M cyclic AMP for 1 h; *d* and 3T3 cytosol incubated with 10^{-4} M db cyclic AMP for 1 h. Activities shown are 3 H-cyclic AMP-binding activity (\square), protein kinase activity in the absence (\triangle) and presence (\bullet) of 10^{-6} M cyclic AMP, NaCl concentration (\cdots), and protein (\circ).

and transformed cells indicated that the cyclic AMP-binding activity from SV3T3 cells was substantially more heat resistant than the binding activity in 3T3 cytosol. Thus, in these studies, thermolability measurements seemed to be a more sensitive probe of differences between species of cyclic AMP-dependent protein kinases than either specific activity or K_d measurements. An increased heat stability of cyclic AMP binding activity *per se* is not a measure of tumorigenicity. The cyclic AMP binding activity of a highly malignant clone of neuroblastoma has been found to be more heat sensitive than that of a less tumorigenic clone¹⁹.

Elution profiles of the cyclic AMP-binding and protein kinase activities after DEAE-cellulose chromatography demonstrated major differences between cytosols from normal and transformed 3T3 cells. 3T3 cytosol contained a type II cyclic AMP-dependent protein kinase whereas SV3T3 cytosol contained both types I and II kinases (see Fig. 2). The fact that the Swiss 3T3 protein kinase elution profile was identical to that of the

BALB 3T3, while the patterns from two different SV3T3 clones were identical with each other, strongly suggests that the different kinase compositions resulted from virus transformation. These results are considerably different from those in which hepatoma cell lines were compared with normal rat liver^{16,20}. In these studies it was the tumour lines which lacked a type I kinase. A comparison of *in vivo* tissues with *in vitro* cells is not always valid, however.

db cyclic AMP treatment of intact CHO cells caused a dissociation of protein kinase holoenzymes²¹, as determined by Sephadex chromatography. DEAE-cellulose chromatography of SV3T3 cytosol following db cyclic AMP treatment of intact SV3T3 cells also indicated that protein kinase holoenzymes had dissociated into regulatory and catalytic components. Two cyclic AMP-binding peaks were observed when either intact SV3T3 cells or SV3T3 cytosol were treated with db cyclic AMP, while only a single cyclic AMP-binding peak was observed with the db cyclic AMP-treated 3T3 cytosol. Types I and II holoenzymes are apparently composed of identical catalytic components, but different regulatory subunits^{2,3}. The elution profile differences between 3T3 and SV3T3 cytosols may be explained by the presence of a single type of 3T3 protein kinase regulatory subunit, while the SV3T3 cells contained two different regulatory subunits. Some oncogenic viruses contain cyclic AMP-dependent protein kinases²². The additional cyclic AMP binding activity in the SV3T3 cell line may have been coded for by the viral genome. Alternatively, an infecting SV3T3 virus may have derepressed the expression of a type I protein kinase regulatory subunit in the host 3T3 cell.

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Partially supercoiled replication intermediates of R plasmid DNA are resistant to relaxation

A NUMBER of bacterial plasmids, including colicinogenic plasmids, F plasmids, and R plasmids have been isolated from *Escherichia coli* in the form of covalently closed circular (CCC) or supercoiled DNA-protein relaxation complexes which can be converted to a nicked circular (relaxed) DNA form by treatment with such agents as SDS, Pronase, or ethidium bromide^{1,2}. The relaxed molecules contain a single nick in a unique strand and at a unique site of the DNA duplex^{3,4}. These nicks occur at the origin of replication of Col E1 and R6K (refs 5, 6). We report here that the R plasmid ROR12 in *Proteus mirabilis* is replicated as a partially supercoiled DNA molecule, as has been observed for replicating molecules of SV40 and polyoma viral DNA⁷, mitochondrial DNA⁸, and the DNA of several plasmids^{5,6,8-10}. Replicating ROR12 molecules have a broad distribution of buoyant densities between the values of the CCC DNA and nicked circular DNA in an ethidium bromide-caesium chloride (EB-CsCl) density gradient. In addition, a substantial fraction

of replicating ROR12 DNA was found to have a density which was less than the value of the nicked circular form. These partially supercoiled replication intermediates of ROR12 DNA retain their supercoiled characteristics even when the DNA is isolated using lysis conditions which yield all of the non-replicating R plasmid DNA as relaxed molecules. This suggests that either replicating ROR12 molecules do not exist in the form of DNA-protein relaxation complexes as do the non-replicating plasmids or, if they do, the replicating molecules are not susceptible to relaxation in these conditions.

The R plasmid ROR12 is a round of replication mutant of the R plasmid NR1 (ref. 11). There are about four times as many copies of ROR12 per chromosome than there are of NR1. When isolated from *P. mirabilis*, the structure of non-replicating ROR12 DNA depends on the conditions in which the host cells are lysed¹². When the cells are lysed in SV buffer at pH 10.2 (0.15 M NaCl + 0.10 M EDTA, pH 10.2), all of the ROR12 DNA is in the CCC form and the DNA is not subject to relaxation by SDS, Pronase, ethidium bromide, or alkaline pH. But when the cells are lysed in SSP buffer at pH 11.0 (0.15 M NaCl + 0.015 M NaH₂PO₄, pH 11.0), all of the ROR12 DNA molecules contain a single nick in a unique strand of the DNA duplex and the majority of the linear strands seem to be attached to a proteinaceous cellular component¹². These findings suggest that

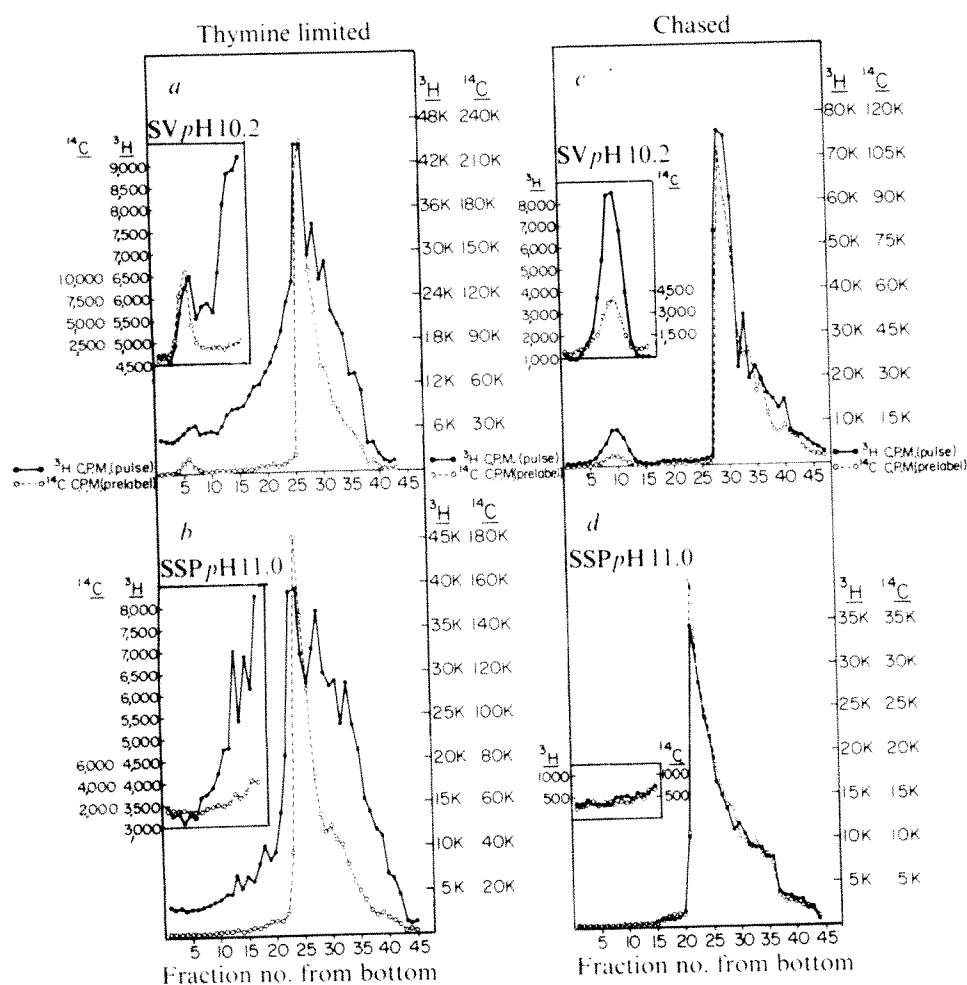


Fig. 1 Density profiles of uniformly labelled and pulse-labelled DNA in an EB-CsCl gradient. Pm15/ROR12 was cultured in M9 minimal medium (supplemented with 0.2% glucose, 10 µg nicotinic acid ml⁻¹, 20 µg tryptophan ml⁻¹, 0.5% vitamin free casamino acids, and ¹⁴C-thymine at 0.75 µCi per 2 µg per ml) at 37 °C with shaking in a New Brunswick Gyrotory shaker bath. At an absorbance, A₆₆₀ of 0.5, 16 ml of culture was filtered on to a 100 mm S and S 0.45 µm membrane filter, washed with 37 °C thymine-free medium, and resuspended in 100 ml fresh 37 °C medium containing ³H-thymine at about 12.5 µCi per 0.03 µg per ml (limiting thymine). After 15 min of incubation at 37 °C with shaking, 50 ml was collected (pulse sample) in ice-cold centrifuge tubes containing ice and sodium azide at a final concentration of 0.01 M. The remaining 50 ml was filtered, washed, and resuspended as above into 50 ml of thymine-free medium at 37 °C. After 5 min (to deplete thymine pools) unlabelled thymine was added to 100 µg ml⁻¹, incubation was continued for 15 min, and the cells were collected as above (chased sample). Half of each sample (pulsed or chased) was washed twice by centrifugation and resuspension in SV pH 10.2 (0.15 M NaCl + 0.10 M Na₂EDTA, pH 10.2) and finally resuspended in 1.0 ml SV. The other half of each sample was washed and resuspended as above except in SSP pH 11.0 (0.15 M NaCl + 0.015 M NaH₂PO₄, pH 11.0). The samples were then lysed by addition of 10 µl 25% SDS, 0.1 ml of Pronase (autodigested, 10 min at 80 °C, 20 mg ml⁻¹) and incubation at 37 °C for 2 h. This was followed by incubation at 65 °C for 30 min and slowly drawing the lysate 20 times into a 1-ml pipette. 0.5 ml of EB (2.5 mg ml⁻¹ in TES; TES = 50 mM each Tris, Na₂EDTA, NaCl, pH 8.0) and 2.09 ml TES were added and mixed gently, followed by 3.45 g of solid caesium chloride. The mixture was centrifuged at 23 °C in a Beckman 50 Tri rotor for 60 h at 35,000 r.p.m. The gradients were collected on to strips of Whatman 3 MM filter paper (1 inch wide with 1-inch squares marked on the strips) through holes punctured in the bottom of the tubes. The strips were washed three times in ice-cold 7.5% trichloroacetic acid and three times in 95% ethanol, and dried in an oven at 160 °C. The squares were cut off the strips into scintillation vials and counted with PPO-toluene (19 gm PPO per gallon toluene). ○, ¹⁴C-pre-label; ●, ³H-pulse label. *a*, Pulse-labelled cells, lysed in SV pH 10.2; *b* pulse-labelled cells, lysed in SSP pH 11.0; *c*, chased cells, lysed in SV pH 10.2; *d*, chased cells, lysed in SSP pH 11.0. Inserts are tenfold magnifications of the CCC and intermediate density DNA regions.

slowly drawing the lysate 20 times into a 1-ml pipette. 0.5 ml of EB (2.5 mg ml⁻¹ in TES; TES = 50 mM each Tris, Na₂EDTA, NaCl, pH 8.0) and 2.09 ml TES were added and mixed gently, followed by 3.45 g of solid caesium chloride. The mixture was centrifuged at 23 °C in a Beckman 50 Tri rotor for 60 h at 35,000 r.p.m. The gradients were collected on to strips of Whatman 3 MM filter paper (1 inch wide with 1-inch squares marked on the strips) through holes punctured in the bottom of the tubes. The strips were washed three times in ice-cold 7.5% trichloroacetic acid and three times in 95% ethanol, and dried in an oven at 160 °C. The squares were cut off the strips into scintillation vials and counted with PPO-toluene (19 gm PPO per gallon toluene). ○, ¹⁴C-pre-label; ●, ³H-pulse label. *a*, Pulse-labelled cells, lysed in SV pH 10.2; *b* pulse-labelled cells, lysed in SSP pH 11.0; *c*, chased cells, lysed in SV pH 10.2; *d*, chased cells, lysed in SSP pH 11.0. Inserts are tenfold magnifications of the CCC and intermediate density DNA regions.

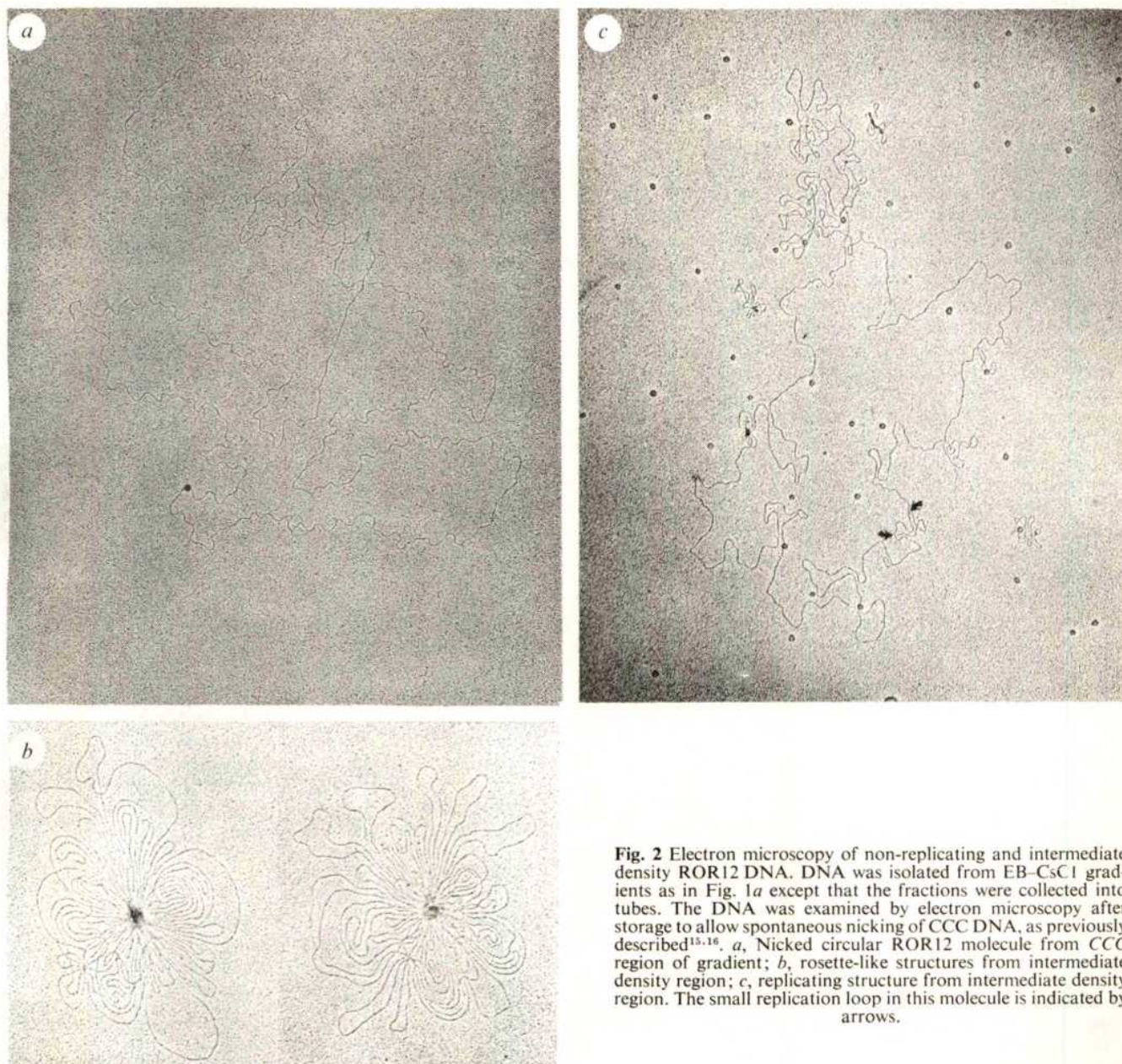


Fig. 2 Electron microscopy of non-replicating and intermediate density ROR12 DNA. DNA was isolated from EB-CsCl gradients as in Fig. 1a except that the fractions were collected into tubes. The DNA was examined by electron microscopy after storage to allow spontaneous nicking of CCC DNA, as previously described^{15,16}. *a*, Nicked circular ROR12 molecule from CCC region of gradient; *b*, rosette-like structures from intermediate density region; *c*, replicating structure from intermediate density region. The small replication loop in this molecule is indicated by arrows.

100% of non-replicating ROR12 DNA exists in the form of a DNA-protein relaxation complex in *P. mirabilis* cells. During lysis in SV buffer at pH 10.2, the protein seems to be dissociated from the DNA which remains in the CCC form. During lysis in SSP buffer at pH 11.0, all of the plasmid molecules are relaxed to the nicked circular form.

In *P. mirabilis* the fraction of plasmid molecules which is in the process of replication can be greatly increased by culturing a thymine auxotroph in medium containing limiting concentrations of thymine¹³. *P. mirabilis* strain Pm15/ROR12 was uniformly labelled with ¹⁴C-thymine and then pulse-labelled for 15 min in medium containing a limiting concentration of ³H-thymine (0.03 $\mu\text{g ml}^{-1}$). A sample of the culture was removed for DNA isolation and, after the addition of a high concentration of unlabelled thymine, the remaining culture was chased for 15 min before isolation of DNA. One-half of both the pulsed and chased samples was lysed in SV buffer at pH 10.2 and the other half of the samples was lysed in SSP buffer at pH 11.0. The lysates were centrifuged to equilibrium in EB-CsCl gradients in order to separate the DNA on the basis of the fraction in the supercoiled form and, hence, the extent of replication.

Figure 1a shows the density distribution of the DNA from

the sample which was lysed in SV buffer at pH 10.2. The ¹⁴C-prelabelled DNA is found in two bands which correspond to CCC plasmid DNA (fractions 4–8) and linear chromosomal DNA (fractions 25–37). A large fraction of the ³H-pulse-labelled DNA has a density between the values of the non-replicating CCC ROR12 DNA and the linear chromosomal DNA (referred to as intermediate density DNA), as has been observed for other replicating circular DNA molecules^{5–10}. In addition, a substantial fraction of the ³H-pulse-labelled DNA has a density which is actually lower than the density of the linear chromosomal DNA (referred to as a light-shoulder DNA). Intermediate density DNA or light-shoulder DNA is not observed in ³H-pulse-labelled DNA from either an R⁻ culture of *P. mirabilis* which has been subjected to thymine limitation or from a culture of Pm15/ROR12 which was not subjected to thymine limitation. DNA hybridisation experiments and buoyant density analysis of purified light-shoulder DNA after removal of EB indicate that the light-shoulder DNA is ROR12 DNA (data not shown). In a reconstruction experiment using a mixture of nicked circular ³H-labelled ROR12 DNA and ¹⁴C-labelled *P. mirabilis* chromosomal DNA, the nicked plasmid DNA had a slightly higher density than the *P. mirabilis* chromo-

somal DNA in an EB-CsCl gradient, as would be expected on the basis of their densities which were determined in an analytical ultracentrifuge (1.712 and 1.700 g ml⁻¹, respectively)¹¹. Taken together, these observations suggest that the light-shoulder DNA is an intermediate in ROR12 DNA replication.

Figure 1b shows the density distribution of the DNA which was prepared from the thymine-limited culture which was lysed in SSP buffer at pH 11.0. In agreement with our previous observations¹², no ¹⁴C-prelabelled non-replicating CCC ROR12 DNA is observed when the cells are lysed under these conditions. The small ³H-labelled CCC band in fractions 4-8 in Fig. 1a is also not observed in Fig. 1b. The ³H-pulse-labelled DNA is, however, still present in the intermediate density and light-shoulder regions when the cells are lysed in SSP buffer at pH 11.0.

The ¹⁴C-labelled and the ³H-labelled ROR12 DNA isolated from the chased culture both band at the position of the non-replicating CCC plasmid DNA in the gradient when the cells are lysed in SV buffer at pH 10.2 (Fig. 1c). The increase in the fraction of plasmid DNA which was replicated during thymine limitation is reflected in the increase in the relative proportion of the ³H-labelled plasmid DNA band. When the chased culture was lysed in SSP buffer at pH 11.0, neither ¹⁴C-labelled nor ³H-labelled CCC ROR12 DNA was observed (Fig. 1d), in agreement with our previous experiments with non-replicating molecules¹². Since the plasmid molecules would have completed replication during the chase period, neither the intermediate density DNA nor the light-shoulder DNA is observed in Fig. 1c and d. The ³H-labelled DNA present in these regions in the DNA from the thymine-limited culture would have been 'chased' into non-replicating CCC molecules to produce the enlarged ³H-labelled ROR12 DNA band seen in Fig. 1c. These CCC molecules would have been relaxed when the DNA was isolated using SSP buffer, pH 11.0 (Fig. 1d).

When DNA from the ¹⁴C-labelled CCC plasmid band of Pm15/ROR12 cultures which have been subjected to thymine limitation is examined by electron microscopy after storage for several days to allow spontaneous nicking of supercoils, most of the DNA molecules are observed to be open circular structures which have contour lengths of ROR12 DNA (Fig. 2a). However, the majority (99/104 in one experiment) of the intermediate density DNA molecules are in the form of rosette-like structures (Fig. 2b). Some open circular structures containing small replication loops are also found in the intermediate density region (Fig. 2c) which have the contour length of ROR12 DNA. Owing to the twisting and overlapping of the molecules, the length of the DNA in the rosettes is difficult to measure accurately, but seems to be similar to that of replicating ROR12 DNA molecules. Rosette-like structures are also found in the DNA from the light-shoulder region. Whereas a minor fraction (8/31 in the experiment shown in Fig. 2) of the DNA from the ¹⁴C-labelled CCC plasmid band of Pm15/ROR12 cultures which have been subjected to thymine limitation appear as rosette-like structures, these structures are rarely observed in the corresponding CCC plasmid DNA band from either chased cultures or from cultures not subjected to thymine limitation. Similar results have been reproducibly obtained in several experiments which indicates that the rosette-like structures are not due to nonspecific aggregation of DNA and proteins under the conditions of these experiments (our work, in preparation).

These results indicate that ROR12 DNA replicates in the form of a partially supercoiled structure and that a substantial fraction of the replicating plasmid DNA actually has a density in an EB-CsCl gradient which is less than observed for the nicked circular form of the plasmid DNA. These replication intermediates remain in the partially supercoiled form during cell lysis conditions (SSP buffer, pH 11.0) which yield all of the non-replicating plasmid DNA as nicked (relaxed) structures. This suggests that whatever is responsible for producing the strand-specific nick in non-replicating molecules of ROR12 DNA under these lysis conditions does not affect the replicating plasmid molecules. Thus, either replicating ROR12 DNA does

not exist in the form of a relaxation complex, or, if it does, the complex is not capable of introducing the single nick into replicating plasmid molecules. Although the rosette-like structures which have been observed in the electron microscope have not been further characterised, it is interesting to note that these structures occur in the same density regions of the EB-CsCl gradient as the partially supercoiled replication intermediates.

The plasmids Colicin E1, R6K, and pSC101 have been isolated from *E. coli* as DNA-protein relaxation complexes¹⁻³. Relaxation of these molecules can be induced by treatment with SDS or EB. Replicating molecules of these plasmids have, however, been isolated from *E. coli* and have a density in an EB-CsCl gradient which is intermediate between the densities of the CCC and nicked circular forms of the DNA^{5,6,8,9}. Although not stated by the authors in these other investigations, these findings suggest that the replication intermediates of these plasmids are also resistant to relaxation by conditions which have been shown to induce relaxation of the non-replicating plasmid molecules. We have also studied the replication of the plasmid RSF2124 (Colicin E1 plasmid containing the ampicillin transposon)¹⁴ in *P. mirabilis* during thymine limitation, and have obtained results which are essentially identical to those described above for ROR12 DNA, including the presence of a light-shoulder replication intermediate.

In the cases of Colicin E1, which replicates unidirectionally, and R6K, which replicates bidirectionally, the site of the nick introduced by relaxation of the complexes has been found to be at or near the origin of replication of each of these plasmids^{5,6,8}. It has been suggested that relaxation complexes may function *in vivo* by acting as a swivel mechanism which allows unwinding of the parental strands during DNA replication¹. Alternatively, the relaxation complexes may provide the first nick which might be required for the initiation of replication. The results presented here (as well as data published by other laboratories) indicate that replicating plasmid molecules are not found in the form of relaxation complexes or that the complexes are not capable of introducing a nick into partially supercoiled replicating molecules. Thus, the function of relaxation complexes may be more probably involved with the initiation of replication rather than as a swivel mechanism during chain elongation, although other possibilities are not excluded.

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Mechanism of DNA synthesis inhibition by arabinosyl cytosine and arabinosyl adenine

1- β -D-ARABINOFURANOSYL CYTOSINE (ara-C) and 9- β -D-arabinofuranosyl adenine (ara-A) are potent antileukaemic and antiviral agents which inhibit DNA replication in various organisms¹⁻³. Two plausible hypotheses for their cytotoxic action centre on the replicative DNA polymerase: in model 1 the triphosphate derivative of ara-C or ara-A inhibits competitively the utilisation of the corresponding deoxyribonucleoside triphosphate in the DNA polymerase reaction, and in model 2 the analogue is incorporated into DNA and this lesion impedes synthesis by, for example, providing a poor primer for further chain elongation. Although both of these effects have been demonstrated *in vitro*, the target *in vivo* for the analogues has not been firmly established, in part because metabolites of these drugs inhibit a number of purified nucleic acid enzymes¹⁻⁴. Bacterial mutants with altered drug sensitivity could be decisive in determining the mechanism of inhibition and are the subject of this report.

Previous studies showed that ara-CTP inhibits replicative DNA synthesis in toluene-treated *Escherichia coli* and *Bacillus subtilis* and the purified DNA polymerases II and III but not I of these organisms⁴⁻⁶. We demonstrated that ara-CTP was also a substrate for the *B. subtilis* polymerases and concluded therefore that DNA chain growth was impeded by an ara-CMP residue at the 3'-hydroxyl terminus of the primer, as postulated by model 2 (ref. 4). Although the crucial enzyme for replication, DNA polymerase III (ref. 7), is less sensitive to ara-CTP than DNA polymerase II *in vitro*, even a low frequency of incorporation by polymerase III would result in the inhibition of propagation of the growing fork *in vivo*⁴. Substantiation of this mechanism by genetic means has been impeded by the inefficient growth inhibition of bacteria as compared to eukaryotic cells by ara-C and ara-A (refs 3 and 8).

We discovered that a DNA polymerase I mutant of *B. subtilis* had a very low efficiency of plating on ara-C or ara-A containing media, whereas the isogenic *polA*⁺ control was essentially unaffected by the highest concentration of drug tested (Fig. 1). (The genetic nomenclature is detailed in refs 4 and 9.) In other experiments, the analogues were shown to be bacteriocidal for *polA* mutants. The sensitisation to drug is caused by the DNA polymerase I mutation. Three independent *B. subtilis* *polA* mutants (*polA59*, *polA13* and *polA06* alleles) were similarly drug sensitive. DNA from a *polA06* strain was used to transform a strain containing the linked *mdh* marker⁴, and 11 of the 77 *mdh*⁺ transformants were *polA* and ara-C sensitive with the

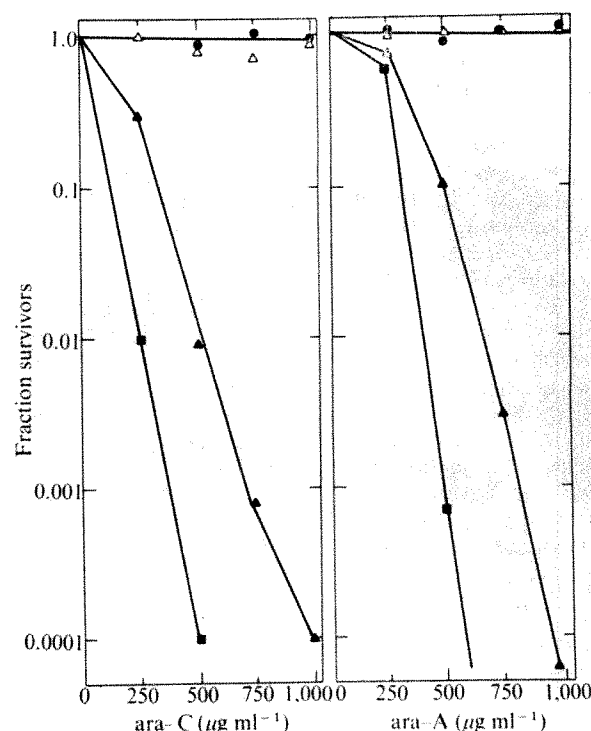


Fig. 1 Efficiency of plating on ara-C and ara-A media. *B. subtilis* strains BD311 (*pol*⁺), ●; BD314 (*polC26*), △; BD315 (*polA59*, *polC26*), ▲; and BD318 (*polA59*), ■; were spread on KML media¹⁰ containing the indicated drug concentration. After 1–2 d at 25 °C, the colonies were counted. The polymerase mutations were introduced by transduction with phage AR9 and strain BD311 is a *pol*⁺ sibling of strain BD318 (D. Dubnau, personal communication).

remainder being *polA*⁺ and ara-C resistant. Introduction of *polA* mutations into several *B. subtilis* strains always conferred drug sensitivity, although the extent of killing was strain dependent, particularly for ara-A.

A *polA1* strain of *E. coli*, H560 (ref. 11), was also sensitive to ara-C. In these experiments, filter disks impregnated with ara-C were placed on KML (ref. 10) agar plates spread with bacteria. Whereas 250 μg of ara-C caused a clearing zone in the lawn of strain H560, a *polA*⁺ isogenic control was not inhibited by even 2 mg of the drug, the highest amount tested. All 280 P1 transductants of strain H560 to methyl methane-

Table 1 Efficiency of plating of *B. subtilis* mutants on ara-C and ara-A containing media

Strain	Relevant genotype	Efficiency of plating on: ara-C	ara-A	Source of strain or ref.
HA101	wild type	1.0	1.0	13
F2	<i>polA59</i>	0.0003	—	13
F22	<i>polA59</i> , <i>polC22</i>	0.03	—	13
F25	<i>polA59</i> , <i>polC25</i>	0.00003	—	4
F26	<i>polA59</i> , <i>polC26</i>	0.09	—	4
F27	<i>polA59</i> , <i>polC27</i>	0.0001	—	4
BD311	wild type	1.0	1.0	D. Dubnau
BD318	<i>polA59</i>	0.0001	0.0007	D. Dubnau
BD191	<i>recB2</i>	0.009	0.1	14
BD191-1	wild type	0.9	0.7	This report
BD193	<i>recD3</i>	0.07	0.1	14
BD193-1	wild type	0.8	0.8	This report
BD194	<i>recA1</i>	1.0	0.7	14
BD224	<i>recE4</i>	1.0	1.0	14
BD246	<i>recG13</i>	1.0	1.0	14
GSY1028	<i>recB2</i>	0.006	0.1	15

The survival on 500 μg ml⁻¹ ara-C or ara-A was measured as in Fig. 1. Since the killing of F2 and its *polC* derivatives by ara-A was low and variable, these data were omitted.

sulphonate resistance (*polA*⁺) were ara-C resistant. The inclusion in the agar of 0.5 $\mu\text{g ml}^{-1}$ tetrahydrouridine, an inhibitor of cytosine deaminase¹², increased the sensitivity of strain H560 fivefold and resulted in the inhibition of the *polA*⁺ control by 250 μg of ara-C.

The enhanced killing of *polA* mutants made it simple to test the prediction of both inhibition models that variation in drug resistance should be conferred by mutational alteration of DNA polymerase III. According to prediction, the plating efficiency on 500 $\mu\text{g ml}^{-1}$ drug for two *polA*, *polC* mutants of *B. subtilis* was about two orders of magnitude higher than for their *polA* parents (Fig. 1, Table 1); this difference was large enough to permit direct selection of *polC* mutants in reconstruction experiments. The other *polC* mutations tested had either no effect or caused increased killing by the drug (Table 1 and unpublished data). The alteration in drug sensitivity is not a result of an outside mutation since the *polC* mutants were selected as spontaneous hydroxyphenylhydrazinopyrimidine resistant mutants^{4,13}, and transfer of the *polC*26 mutation to a *polA*59 strain by transduction to form strain BD314 resulted in resistance to ara-C and ara-A (Fig. 1).

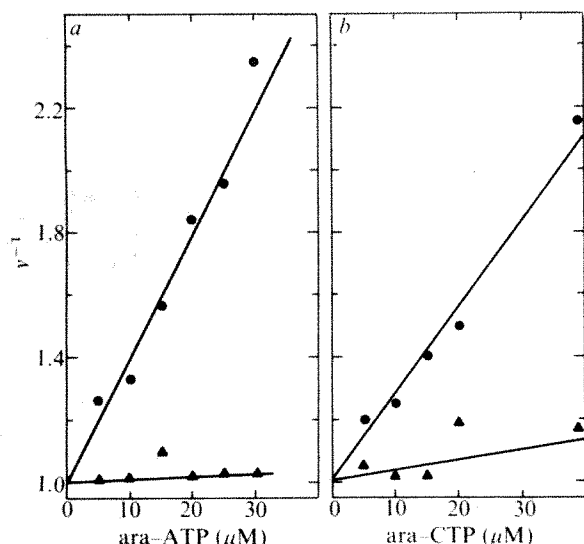


Fig. 2 Inhibition of wild-type and mutant DNA polymerase III by ara-ATP and ara-CTP. The reaction conditions were as described¹⁶ except the time of incubation at 30 °C was extended to 90 min; in *a*, the dATP concentration was 5 μM and ara-ATP was added to the indicated level, and in *b*, the dCTP concentration was 5 μM and ara-CTP was added to the indicated amounts. Each reaction contained 4 munits of wild-type, ●, or *polC*22, ▲, DNA polymerase III purified from *B. subtilis* strains BC26(F) and F22, respectively^{12,16}. Since synthesis truncated¹ by omission of dATP was not inhibited by ara-ATP and that truncated by omission of dCTP was immune to ara-CTP, these values were subtracted in *a* and *b* respectively. The resultant reaction rate, *v*, in the absence of drug was set equal to 1.0. The calculated *K_i* values for the wild-type and mutant enzymes are 4 and 150 μM for ara-ATP and 2 and 17 μM for ara-CTP.

The *polC*22 DNA polymerase was shown to be intrinsically drug resistant. The purified mutant DNA polymerase III was about an order of magnitude more resistant to both ara-CTP and ara-ATP than the wild-type enzyme (Fig. 2). *A priori*, the drug resistance could be the result of either an increase in the inhibition constant (*K_i*) for the drugs or a decrease in the *K_m* for the competitor deoxyribonucleoside triphosphate. Since we showed previously that the *K_m* for dGTP was the same for the *polC*22 and wild-type polymerases¹³, and the *K_m* for dCTP was measured to be 0.3 μM for both the enzymes used here, the increased resistance of the mutant enzyme results from an increase in the *K_i*. The mutational alteration of the *polC*22

DNA polymerase near the active site¹⁷ must create a negative interaction with a portion of the arabinosyl compounds and hydroxyphenylhydrazinopyrimidines which is not shared by deoxyribonucleoside triphosphates. By analogy with the mechanism of resistance of the *polC*22 polymerase to the arylhydrazinopyrimidines¹⁷, it is likely that this polymerase incorporates the ara-NTP residues inefficiently, rather than excising ara-NMP termini more rapidly or using these residues more effectively as primers.

The drug sensitivity conferred by the *polA* mutation could be a consequence of the role of polymerase I in the replication or repair of DNA⁷. Several other repair-deficient mutants of *B. subtilis* showed no sensitivity to ara-C and ara-A, but *recB*2 and *recD*3 mutants had a reduced plating efficiency on drug-containing medium (Table 1). The drug sensitivity is due to the *rec* mutations. Strain BD193 was constructed by introduction of the *recB*2 mutation of GSY1028 and both strains were analogue sensitive (Table 1). Transduction mediated by phage AR9 of strains BD191 and BD193 to mitomycin C resistance or methyl methanesulphonate resistance rendered the resultant strains (BD191-1 and BD193-1) resistant to the drugs (Table 1). Although it is possible that these genes affect other cellular processes, the simplest conclusion is that the lesions in DNA caused by ara-C and ara-A can be repaired by pathways involving the *polA*, *recB*, and *recD* genes.

Several conclusions can be drawn from the data presented here. First, ara-A and ara-C probably act by way of the same mechanism, since mutations can cause a parallel increase or decrease in sensitivity to these drugs. Second, the marked effect of DNA polymerase mutations implies that metabolism of ara-A and ara-C to the triphosphate level is required for inhibition. Third, the correlated resistance *in vivo* and *in vitro* as a result of a mutational alteration of DNA polymerase III provides the best evidence so far that this enzyme is a primary target for the drug in the cell. Fourth, the simplest explanation of the enhanced sensitivity of *polA* and *rec* mutants is that the drug-induced lesion can be repaired at the DNA level, as required by model 2 but not by model 1.

This work was supported by grants from the NIH. We thank D. Dubnau for constructing some of the bacterial strains and the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute for the gift of tetrahydrouridine.

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matters arising

Possible mechanism for biological action of lithium

FRAUSTO DA SILVA and Williams¹ have presented a theoretical chemical rationale for the postulate that lithium may have its pharmacological action by competition with magnesium²⁻⁴. But though they have dealt in detail with the role of ATP as a "masking" agent they have not considered the direct binding of lithium to the nucleotide.

Since lithium might interfere in the many biological processes which are magnesium dependent⁵ and since changes do occur in the tissue distribution of magnesium following lithium⁶ my colleagues and I investigated five magnesium-dependent enzymes and found that three were inhibited by lithium⁷. These enzymes seemed to be "Mg-ADP dependent".

We determined, by gel filtration on Sephadex G-10, the association constants of lithium and magnesium with either ADP or ATP both independently (K_i) and also in a solution containing both metals⁸. The constants determined in the (Li+Mg) solution were calculated *a*, assuming that the nucleotide complexed Li and Mg independently (K_{ii}) and *b*, assuming that the metals competed for sites (K_{iii}). All experiments were carried

Table 1 Association constants (log K_{ass}) for complexes of Mg and Li with ADP+ATP in conditions described in the text⁹

		ADP	ATP
K(i)	Mg	3.02 (a)	3.97
	Li	2.48 (b)	2.71
K(ii)	Mg	3.12 (c)	4.00
	Li	3.00 (d)	2.79
K(iii)	Mg	3.22 (e)	4.05
	Li	3.14 (f)	3.07

By *t* tests the following pairs are significantly different at $P < 0.001$: (a) against (e), (b) against (d), (b) against (f).

out at pH 7.4 in 0.108 mol l⁻¹ triethanolamine hydrochloride buffer. The results are shown in Table 1.

Our values for K_{ass} differ from those of Frausto da Silva and Williams but agree with other reports⁹⁻¹⁰. But they are effectively "conditional constants" with respect to the nucleotide-buffer-metals system at pH 7.4. We have concluded that there is preliminary evidence for a ternary complex of the Li-Mg-ADP type⁸ though this awaits confirmation by physical methods.

In relation to magnesium, ADP has a rather higher affinity for lithium than

ATP and if the lithium complex were a poor substrate this might have implications in the kinetics of ATPases during lithium therapy. In any case the increased binding of lithium induced by the presence of magnesium must indicate configurational or other changes which might affect the kinetics of any ADP requiring enzyme.

Our findings with biologically occurring nucleotides therefore suggest that the sequestering agent (ATP) may also be affected by lithium due to effects on the ATP-ADP equilibria. We might expect two loci of lithium action in nucleotide dependent enzymes; (O⁻, N, O⁻, O⁻) coordinating sites on the protein and the nucleotide itself.

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WILLIAMS AND FRAUSTO DA SILVA REPLY—We accept the points made by Birch. The major point of our paper was to show how lithium could act on vesicles which contained transmitters even in situations where magnesium ions might be thought to be protective against any lithium effect.

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Uniqueness of plasminogen activators

THE report of Åstedt and Holmberg¹ relating their demonstration of the immunological identity between human urokinase and plasminogen activator from human ovarian carcinoma includes a quotation from the summary of one of our papers on plasminogen activators from transformed cells². Because of the interest in the role of plasminogen activators in tumorigene-

sis, we feel that it is important that there is no misinterpretation of our statement: "Neoplastic cells, whether transformed by oncogenic viruses or chemical agents, release a fibrinolytic factor not released by normal cells". In the text of our article, this sentence is qualified to indicate that by 'normal cells', we mean the parental primary cultures from which the transformants were derived. Our purpose in characterising the plasminogen activator from a transformed line was to determine whether or not it differed from the plasminogen activators known to be produced by normal mammalian tissues. We have, in fact, published data which demonstrate that the plasminogen activator which we purified from SV40 transformed hamster cells is immunologically identical to that produced by normal hamster lung cells and that it differs from the plasminogen activator(s) produced by hamster kidney cells³.

Although there is no doubt that there is a tantalising correlation between plasminogen activator production by cells *in vitro* and their ability to cause tumours in immune-competent hosts^{4,5} or in nude mice⁶, we feel that it is worth stressing once again that not all cultured cells which produce plasminogen activator are tumorigenic, nor do all cells capable of forming tumours in immunocompetent hosts produce plasminogen activators *in vitro* (for example, Friend erythroleukaemia cells). In addition, as is confirmed by the work of Åstedt and Holmberg, the plasminogen activators produced by tumour tissues are not 'tumour-specific' proteins but probably reflect the expression of genetic information in 'inappropriate' cells.

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Temperature sensitive defect in human-hamster cell hybrid

In a recent report Talavera, Basilico and Croce¹ reported incomplete complementation of a temperature-sensitive defect in 28S ribosomal RNA (rRNA) maturation after hybridisation of a Syrian hamster line, BHK 21/13 *ts422E* and LW SV40 transformed human cells. The incomplete complementation was expressed as a significant decrease in the growth rate of the hybrid cells as well as a reduced level of 28S rRNA production. In three of the hybrid populations tested, one contained exclusively BHK 28S rRNA and the other two showed a distribution of greater than 90% BHK 28S rRNA and only a very small proportion of human 28S rRNA. These results contrasted markedly with the situation with hybrids of the same BHK *ts422E* cells and two mouse lines in which good complementation was observed with the production of both mouse and hamster 28S rRNA at the non-permissive temperature (39 °C)². The distribution of mouse and hamster 28S rRNA ranged from predominantly mouse to predominantly hamster with some hybrids showing an equal distribution of both

those utilising pathway *b*, such as human cells. Furthermore, assuming that the BHK *ts* cells at 39 °C cannot provide all of the products necessary for maturation by pathway *b*, one could also account for the low yields of human 28S rRNA in the two hybrids analysed by Talavera *et al.*¹, which are presumed to contain actively transcribing human rRNA genes. Since the BHK *ts422E* mutant at the permissive temperature (33 °C) uses predominantly pathway *b*, characteristic of human HeLa cells^{3,4}, our interpretation leads to the testable prediction that at 33 °C *ts422E* should be able to provide the gene products necessary for human 28S rRNA production in the BHK × human cell hybrids F3 and F31.

The alternative explanations offered by us and by Talavera *et al.* are based on two different assumptions about the nature of the complementing function supplied by the L and HeLa cell partners in the hybrids with BHK cells. Given that the temperature-sensitive lesion in *ts422E* is pleiotropic in affecting both the cleavage at site 4 and the relative propensity for cleavage at sites 2 and 3, it is conceivable that the lesion is one which affects the conformation of the preribosomal particles in the

ing pathway in the hybrid cells.

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¹ Talavera, A., Basilico, C., and Croce, C., *Nature* **259**, 667–670 (1976).

² Toniolo, D., and Basilico, C., *Nature*, **248**, 411–413 (1974).

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⁴ Winicov, I., *J. molec. Biol.*, **100**, 141–155 (1976).

TALAVERA ET AL. REPLY—The appearance of 36S as well as 20S nucleolar RNAs in wild-type BHK (refs 1 and 2) indicates that the two alternative pathways of ribosomal RNA (rRNA) processing described by Winicov and Perry^{3,4} are probably an effect of differential rates of cleavage at the sites 2 and 3 (Fig. 1 of ref. 1). Pathway *B* could be absent in *ts422E* at 39 °C because of a partial effect on site 3 of the lesion that blocks completely the cleavage at the neighbouring site 4 (ref. 2). This would favour cleavage at site 2, resulting in a shift to pathway *A*. We therefore believe that pathway incompatibility¹ alone cannot be taken as an explanation of the imperfect complementation found in hybrids between human cells and the BHK mutant *ts422E* (ref. 5).

Wt BHK cells as well as the mutant at 33 °C follow preferentially pathway *B* (characteristic of human cells). Thus, a perfect complementation of the *ts* defect in the human × *ts422E* hybrid should produce both at 33 °C and 39 °C the use of pathway *B*, which is the preferred pathway of both parent cells.

On the other hand the low amount of human 28S rRNA found at 39 °C in the hybrid populations F3 and F31 (ref. 5) can be explained according to Winicov and Perry⁴ only if one assumes that human rRNA processing functions are lost in the hybrids, as the parental human cells are not defective in rRNA processing. This seems unlikely; even if it cannot be completely excluded. As already discussed⁵, the selection and propagation of the hybrid lines should have strongly favoured cells which maintained the human chromosome(s) necessary to attain the highest possible level of 28S RNA.

For these reasons we believe that the defective cross-specific rRNA processing in our hybrids is more likely to be caused by specific binding differences in the formation of preribosomal particles, rather than by incompatibility between different processing pathways.

¹ Winicov, I., *J. molec. Biol.*, **100**, 141–155 (1976).

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⁴ Winicov, I., and Perry, R. P., *Nature*, **264** 682 (1976).

⁵ Talavera, A., Basilico, C., and Croce, C., *Nature*, **259**, 667–670 (1976).

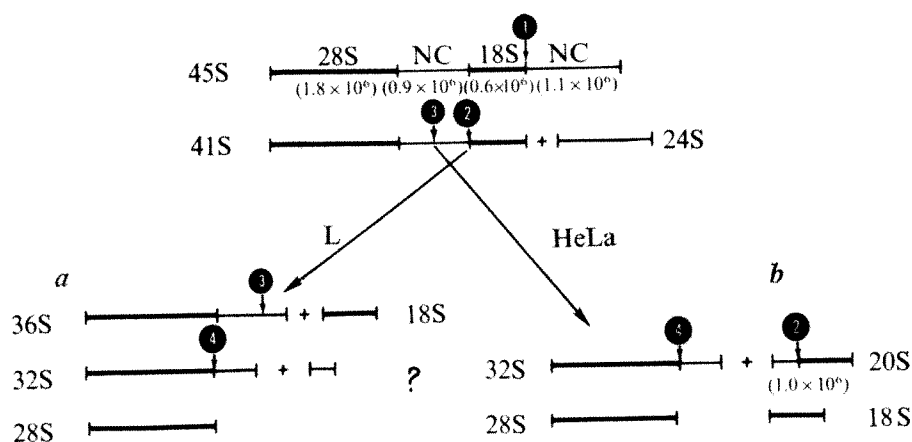


Fig. 1 The two pathways by which BHK *ts422E* can process rRNA.

28S rRNA types. We would like to offer a possible explanation for the poor complementation observed in the BHK human hybrids based on our own published data^{3,4}.

BHK *ts422E* can process rRNA by two alternative pathways as shown in Fig. 1: pathway *a*, which is characteristic of mouse L cells, and pathway *b*, which is characteristic of human HeLa cells. The BHK *ts422E* mutant at its non-permissive temperature (39 °C) uses pathway *a*, which in this case is defective at the 32S → 28S step. In hybrids of *ts422E* one might therefore expect complementation by gene products from cells exhibiting pathway *a*, such as mouse cells, but not from

regions of the relevant cleavage sites. The uncertainty is whether the complementing function restores the order of cleavages to pathway *b*, characteristic of the wild-type BHK cells, or whether the complementing function simply enables the *ts422E* cells to utilise pathway *a* more productively. The only essential feature of the complementation that was observed in the hybridisation experiments is that it restored the ability to make a proper cleavage at site 4, which is required for viability at the non-permissive temperature. Whether the other consequences of the pleiotropic lesion are also complemented cannot be ascertained without a more detailed analysis of the process-

reviews

Black hole, meteorite or spacecraft?

W. H. McCrea

The Fire Came By: The Riddle of the Great Siberian Explosion. By J. Baxter and T. Atkins. Pp. 165+39 plates. (Macdonald and Jane's: London, October 1976.) £3.95.

ON June 30, 1908, there took place in a remote subarctic region of Siberia the event that for many years was regarded as the greatest meteoritic fall ever recorded. The general character of the occurrence was that of a stupendous explosion, almost certainly in the nature of an 'air burst'. For some decades now increasing doubt has been cast on the meteoritic hypothesis. As is well known, almost nothing has been too fantastic to have been proposed as an alternative. This book rehearses the story and the puzzle presented by it, and the authors advance their version of a particular class of solution.

News of the happening was slow in reaching the world at large and it made little impression when it originally did get abroad. The scientific community became aware of it only because an old newspaper report came to the attention of L. A. Kulik of the Mineralogical Museum in Petrograd (Leningrad) when he was preparing an expedition to locate meteorites that had fallen in USSR territory. After preliminary investigations in 1921, he and an assistant made an heroic exploration of the region in 1927, followed by several later expeditions. Much of what is known about the observable physical effects and of what was gleaned from surviving contemporary accounts and from the recollections of surviving witnesses we owe to this work. But there have also been Soviet ground and aerial surveys right into the 1960s. Many scientists in the USSR and elsewhere have worked on the interpretation of the evidence. All this is vividly described in the first half of the book, numerous photographs are reproduced and the appendices supply translations of reports of two expeditions.

The authors then discuss the problem that arose after it was generally appreciated that the evidence was incompatible with the fall of any known sort of meteorite. As they recall, as long ago as 1930 the English mathematician and meteorologist (not astronomer, as



Leonid Kulik, chief Soviet investigator.

stated), F. J. W. Whipple, and a few years later the Russian I. S. Astrapovich, suggested that the object concerned was a small comet. This remains the most generally favoured explanation, and the authors dismiss it much too casually. If the hypothesis is modified by supposing the object to have been a fragment of a comet that disintegrated at about the Earth's distance from the Sun, as comets are apt to do, and not just a mini-comet all on its own, then it does indeed appear as much the most plausible of the proffered explanations.

The authors allow themselves to be beguiled by the two speculations: that a spacecraft was concerned and that the explosion was atomic. That the explosion was such they contend from a comparison of its effects on the ground and in the atmosphere with those of atomic bombs exploded in 1945 and in tests before and since. For fairly generally accepted reasons, they discount the alternatives of an explosion caused by a body of antimatter or a black hole. As scarcely more than science fiction, the suggestion that an extraterrestrial spacecraft was involved has been around since about 1946. The authors take it seriously because there were reported sightings of a cylindrical missile-like object just before the explosion which, they think, implied two

abrupt changes of course, and because they think the conformation of the devastated area to indicate that the explosion came out of a "non-explosive shell". They go on to suggest that the explosion was a mishap to an atomic-powered spacecraft from some planetary system other than the solar system (although they seem ambiguous about this). The speculation seems to be irresponsible and to be suggested by the flimsiest of evidence. So it cannot be treated as a starting point for serious discussion of the plausibility of communication with extraterrestrial communities.

As is so often urged, we ought not to scoff at the general notion of some form of such communication, and we have to recognise how potential communicators could be physiologically, technologically, psychologically and in almost every other way different from ourselves. On the other hand, any speculation on the subject must proceed on the hypothesis that such beings would be restricted to matter the same as ours, obeying the same laws of physics. But then the concept of something approximately like an interplanetary space vehicle, using atomic power, being used for interstellar excursions is surely quite ridiculous.

This reviewer regrets having to make such remarks about a book on this amazing event that starts so well. Having accumulated so much fascinating material the authors could have sifted it into hard observations made on the scene by professional scientists, seismic and meteorological observations made elsewhere at the time and the reasons for associating these with the event, and the adequately corroborated elements in eye-witness accounts. They seem also to have the material for proceeding to some quantitative comparison of this evidence with the predictions of the various theoretical models that have been proposed. Had the authors done so—I cannot but think they would have reached very different conclusions—their book would have made even better reading than it does now, and it would have been a unique permanent contribution. □

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Dynamic panorama of astronomy

Our Changing Universe: The New Astronomy. By John Gribbin. Pp. 160. (Macmillan: London, 1976.) £4.95.

ASTRONOMY, in the doldrums thirty years ago, is now a thriving science, with a continuing march of discoveries that have transformed the static picture of past decades into an explosive and dynamic panorama. The immense changes in the science have been wrought by the development of the invisible astronomies that utilise observations in the non-visual ranges of the spectrum. Radio telescopes were the first to ruffle the calm waters of the optical astronomer, and these have been followed by X-ray satellites and a host of observation techniques based on the particle philosophy of modern physics. We now view a new universe, and one which Dr Gribbin sketches succinctly in his admirable book.

Beginning with radio astronomy, pulsars and quasars, we move on in a series of short chapters to cover the whole gamut of the modern scene—X-ray and neutrino astronomy, the significance of cosmic ray observations, the existence or otherwise of gravitational waves, the cosmic background radiation, and the question of whether the Universe is of the big bang, oscillating, or some altogether different type. In fourteen brief chapters covering some 66 pages of text, we have a brief but concise survey of the stellar and galactic universes.

There is also a chapter on the planets in the light of recent space probe observations, and another where Dr Gribbin gives his own views on what astronomy may be doing in the near future—a section where optical astronomy comes in for some comment. Finally there is an appendix, "Ice Ages, Man and Solar Neutrinos", in which there are some controversial ideas on a subject that is one of the author's consuming interests.

To concentrate most of the text on new observation techniques and their results was wise since they provide an ideal subject for a brief survey designed to be both relevant and exciting. The book is helped by a great number of well-chosen illustrations, some in colour, and all relevant to the text. There is an adequate index and a useful if restricted bibliography.

Of course, every reviewer is bound to have reservations on how an author apportions his space in a book of this kind—possibly more could have been said about BL Lacertae objects, on the electronic observing techniques now

used on optical telescopes, and at least a passing reference to that ingenious invention, the intensity interferometer—but there are bound to be personal preferences.

One wonders here and there about the level of previous knowledge expected of the reader: N-galaxies are mentioned but not specifically defined, spectroscopic principles are taken for granted, yet elsewhere the text could be understood even by a raw

beginner. Yet the general approach is such that no-one will get lost even though an equation appears as an integral part of one piece of descriptive writing. Every reader is bound to be swept along by Dr Gribbin's style, which is as dynamic as the universe he describes.

Colin A. Ronan

Colin Ronan is Editor of the Journal of the British Astronomical Association.

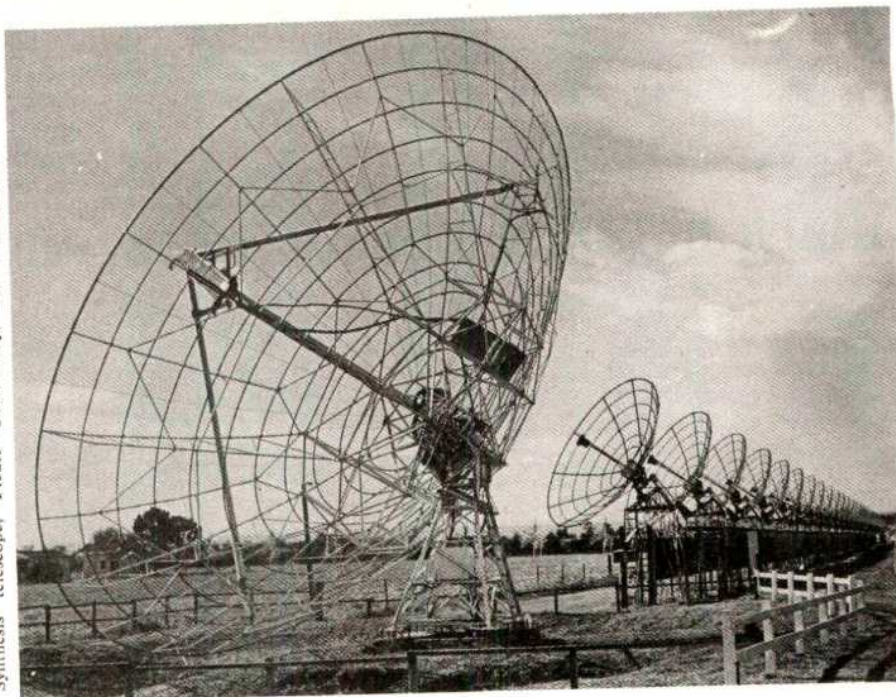
Basic guide to the galaxies

Exploring the Galaxies. S. Mitton. Pp. 206. (Faber and Faber: London, 1976.) £4.75.

THERE are three main readerships for astronomy books: the professional astronomers, the amateur with a serious interest who makes his living elsewhere, and the more casual reader who has a passing (if sometimes quite deep) interest and wants to know the story of what is going on without being swamped by technicalities. Since it is rare that a book can cater satisfactorily for any two of these markets, let alone all three at once, it is well for an author to have his intended readership clearly in mind, and Mitton's *Exploring the Galaxies* is described on the cover as intended for the non-specialist, a "guided tour . . . which brings the reader to the threshold of contemporary research". So this is definitely not a book for the professional astronomer, who has already progressed beyond those fringes, and given its sober design

and the inclusion of diagrams of the kind familiar to readers of *Nature* (indeed, some culled from these pages) seemed therefore aimed at the 'serious' amateur, rather than the casual reader best hooked by pretty pictures. Unfortunately, the author has failed to distinguish clearly between these two markets, with the result that his text steers a sometimes uneasy course between superficiality and technicality, dropping anchor in neither port.

The book certainly starts simply, with a potted account of the history of astronomy—but it's not another account of this history that a prospective reader looks for in any new book on galaxies. Finding his feet with a chapter on instrumentation (but sadly dismissing X-ray telescopes in one paragraph) Mitton gets to grips with the basics of galaxies, their structure and the distances to them at a level which assumes some knowledge of physics, if only at the school rather than university level. At his best in describing recent work on giant radio sources, 'tadpole' radio galaxies and radio studies of QSOs Mitton falls down somewhat on the grounds of readability, producing such tortuous con-



Taken from *Our Changing Universe*.

structions as "it can be seen that at the present time it is not known with any certainty . . ." where a simple "we don't know . . ." would do. I was also disappointed to find very little stress placed on the increasing evidence of links between QSOs and active galaxies.

Overall, the book would be of interest to its intended market, but as such it must face comparison with such accepted standard works as Shapley's *Galaxies* (revised by Paul Hodge only a few years ago), Baade's *Evolution of*

Stars and Galaxies and *The Milky Way*, by Bok and Bok. As a basic guide to galaxies for someone with an interest in astronomy, Mitton's book has no major flaws; unfortunately, however, when looked at against the opposition it is top of the second division rather than a big league competitor.

John Gribbin

John Gribbin is a Visiting Fellow at the Science Policy Research Unit, University of Sussex, UK.

Sense of time

An Inventive Universe. By K. G. Denbigh. Pp. 220. (Hutchinson: London, 1975.) £3.75.

DR DENBIGH, Principal of Queen Elizabeth College, University of London, is a scientist who has managed to fulfil the duties of his post without abandoning his own academic activities. A distinguished thermodynamicist he has long had a profound interest in the subject of time, both in its philosophical and its scientific aspects. He has read widely and thought deeply about it and other fundamental concepts in science. Since he also has the gift of writing clearly and concisely, his latest book, which is addressed to a wider audience than most of his previous publications, deserves a warm welcome and it is to be hoped that many will read it.

Denbigh believes, in the widest sense of the term, in the evolutionary nature of the universe. Although he never refers to A. N. Whitehead specifically, he can be regarded in this respect, at least, as one of his followers. Whitehead maintained that our whole conception of knowledge has been vitiated by the assumption, going back to the Greeks, that transition and creation are 'inferior' to changelessness and invariance.

In Denbigh's short book there are five chapters of about 175 pages in all, supplemented by some 35 pages of notes and references. The first, and longest, chapter is on our construction of the concept of time, which he points out depends specifically on mental processes, despite its intersubjective character. Much of physics, however, is based on what he calls "a sort of pared down time concept", involving only the notion of a linear sequence and a quantitative scale. Consequently there is considerable divergence of opinion concerning the anthropomorphic nature, or otherwise, of time's arrow and time's transience. Although Denbigh recognises the inadequacy of the B-theory standpoint (to use McTaggart's well-known terminology), which denies objectivity to the distinctions we make

between past, present and future, he takes the A-theorist Hans Reichenbach to task for using Heisenberg's uncertainty principle as the basis of an objective 'now'. Denbigh's own leanings are towards the A-theorists and he repudiates the claim of those B-theorists who cite relativity in their support, since there is nothing in relativity that *necessitates* the B-theory standpoint. He concludes that the sense of time which we construct out of our conscious awareness is not confined to ourselves but provides an important link between the mental and the physical.

Chapters 2 and 3 are devoted, respectively, to dissipative processes and 'formative' processes. The former are associated with the law of increasing entropy, and the latter with the evolution of organisms and tissues of increasing complexity. These two distinct trends in nature are not incompatible for, whereas entropy is concerned with orderliness (and its decay), biological evolution is concerned with organisation. To elucidate the difference between these ideas Denbigh introduces the concept of "integrability", which he suggests may be analysed mathematically with the aid of graph theory. It is to be hoped that he will eventually find time to develop this stimulating train of thought more fully.

In Chapters 4 and 5, Denbigh discusses determinism and whether genuinely new things can come into existence during the course of time. He is a firm believer in the continual and natural evolution of novelty. At the end of the book he returns to the concept of time, stressing both its relation to consciousness and its intersubjective character. He argues that the totality of consciousness in the universe tends to increase, and he believes that a kind of principle of 'complementarity' (although he does not call it that) between the concepts of physics and chemistry and those pertaining to ourselves as 'persons' (rather than 'things') must be invoked if we are to understand the role of man in the world-order.

G. J. Whitrow

G. J. Whitrow is Professor of the History and Applications of Mathematics at Imperial College, University of London, UK.

Immunological cookery

Practical Immunology. By L. Hudson and F. C. Hay. Pp. xvi+298. (Blackwell Scientific: Oxford, London, Edinburgh and Melbourne, 1976.) £6.50.

THIS book is required in the laboratories of all those who practise the mysteries of contemporary experimental immunology. It is clearly written, adequately illustrated and very useful.

The authors are research workers who have, in addition, considerable experience of teaching both practical and theoretical immunology. It seems from their book that they enjoyed what they were doing, that they did it well and wished to communicate their enthusiasm and competence to others.

In the contents list to the book there are a series of rather arbitrary headings such as "Initial preparations", "Cellular dynamics *in vivo*", "Antibody interaction with antigen", and "Advanced techniques in cellular immunology". But in fact the authors have compiled a list of the main ingredients of immunological cookery and then gone on to provide a series of recipes for their use. The emphasis is avowedly on the cellular components of immune responses as the authors believe that this represents a good starting point from which consideration of the humoral aspects of immunity can develop.

The title of the book is misleading in that it seems to embrace the whole practice of immunology, whereas it is restricted almost entirely to description of the practicalities of avant-garde experimentation. Thus there is hardly any consideration given to parasite immunology except, surprisingly, the Wasserman test; nor to histopathological evaluation of results.

This book, held in conjunction with that on *The Immune System: a Course on the Molecular and Cellular Basis of Immunity* by Hobart and McConnell (Blackwell, 1976) and the older title *Essential Immunology* by Roitt (1974) should equip any budding immunologist with a knowledge of what has been done, how it was accomplished and what it all means. All that would be required to complete this paper armamentarium would be a text on "What to do next in Immunology". That would be useful.

A. J. S. Davies

A. J. S. Davies is Professor of Immunobiology in the University of London, working at the Chester Beatty Research Institute, London, UK.

Trends in Soviet science and design

Soviet Science, Technology, Design: Interaction and Convergence. By Raymond Hutchings. Pp. xii+320+8 plates. (Oxford University: London and New York, May 1976. Published for the Royal Institute of International Affairs.) £12.

THE idea of analysing the evolution of the interactions of science, technology and design in the Soviet Union is interesting and worthy of research, but the present study by Dr Ramond Hutchings must be regarded as a rather idiosyncratic first attempt. His book contains some factual material, notably on design, which will be new to most readers, and a number of suggestive insights into aspects of Soviet technological and design policy. But substantial sections contribute little to the existing literature in English on Soviet science and technology and, more seriously, the study is weakened by inadequacies of a conceptual and theoretical order.

Dr Hutchings discusses science, technology and design in isolation and then concludes with a brief consideration of their interrelationships. Chapters are devoted to the organisation and planning of science and the trends in expenditure on research and development in the post-war years, covering aspects of Soviet science treated in greater detail elsewhere, notably in the OECD study *Science Policy in the USSR* (1969). The chapter on finance is particularly sketchy and takes no account of the valuable studies of Louvan Noltling of the US Department of Commerce. Consideration of some strands of technological policy is followed by a brief account of the problems of technical innovation. In a chapter on 'design as an institution' it is argued that following early interest symbolised by the activity in the 1920s of VKhUTEMAS (the Higher Technical Artistic Studios), a Soviet equivalent of the Bauhaus, interest in design suffered neglect until the early 1960s when a new specialised institute, VNIITE (All-Union Scientific Research Institute of Technical Aesthetics), was established. In describing this interesting organisation the author draws on his direct personal experience.

Finally, chapters are devoted to characteristic features of design in the USSR and the forces which have conditioned them—for example, the well known bias towards grandiose structures, and the influence of ideological symbolism. It is in this section that the author's rather idiosyncratic and eclectic

approach is most apparent, although at times his speculations on Soviet design peculiarities are stimulating and amusing. There are some minor factual inaccuracies, in part stemming from the fact that developments in the past five years are treated less thoroughly than for the 1960s.

The main thesis of the book is that science, technology and design in the Soviet Union are beginning to interact with increasing force in spite of pressures, primarily political and ideological, tending to maintain their relative separation, and that the forms of interaction are showing a tendency to converge with those typical in the West. Moreover, design, it is argued, has until recently, been the weakest link in the chain. Although much evidence in support of these propositions is adduced, there is a notable absence of serious theoretical treatment of the general processes of interaction of science, technology and design under capitalism and socialism and at different stages of industrial development. The concepts 'technology' and 'design'

are themselves defined with insufficient rigour; the former is used loosely to embrace technical sciences, engineering and technical artefacts, whereas 'design' in the main corresponds to activity in the sphere of aesthetics, rather than engineering design (although the author at times slides from one to the other). Thus, consideration of design focuses on VNIITE and little attention is devoted to the extensive network of design and project offices which forms a vital component of the Soviet research and development system. More rigorous treatment of the theoretical framework of this study would have made it a more weighty contribution to our understanding not only of Soviet society, but also of the exciting implications arising from the general tendencies of development of science, technology and design in the second half of the twentieth century.

Julian M. Cooper

Julian Cooper is a Research Fellow at the Centre for Russian and East European Studies, University of Birmingham, UK.

Notions of mechanism in biology

The Problem of Life: An Essay in the Origins of Biological Thought. By C. U. M. Smith. Pp. xxiv+343. (Macmillan: London and Basingstoke, May 1976). £10.

THIS is a gallop through biological history with an eye open for certain views of the passing countryside. It might have been called *The Triumph of Reductionism*, and our author plots the course of notions of mechanism in biology from ancient times to the present. The views of the countryside that attract him are those that show how man's idea of nature is determined by the society in which he lives. 'Biology' had a greater meaning when self-moving things were thought to be alive, or when the Greek natural philosopher, living in a *polis*, used 'political' explanations of the behaviour of animals.

The growth of technology provided another analogy with which to explain biological phenomena, and we are presented here with a biologist's view of the evolution of technology, mutations being selected for and against; such evolution produces a certain kind of society, and the society produces ideas of a certain sort, including those of the natural world. It is certainly

true that the history of science is largely the history of the knack of asking answerable questions of nature. This does not invalidate the unanswered questions, but the answered questions certainly help to mould the mind of the enquirer; this generates a bias in the new questions and the new people who ask them. To a certain extent science and perception of nature are thus 'culture bound', but very often the historian finds that scientific ideas are transmitted through societies, not generated by them, as the biologist or politician may be tempted to think.

The author's awareness of the limits of perception, of the urge of the mind to impose patterns on the perceived, and of the orientation of perception by culture leads him into early chapters on imagination, anthropology and language that are somewhat thin and not entirely related to the rest of the book. The level of scholarship in a book of such broad scope inevitably falters in places. In particular anatomy and Vesalius are inadequately dealt with, and there is some misinterpretation of the position of Borelli and the later animists. Nevertheless, the author's view of history is held with a firmness that provides a coherence and freshness that are particularly valuable in a survey of such a long historical period.

R. French

Dr French is a Lecturer in the Wellcome Unit for the History of Medicine, Department of History and Philosophy of Science, University of Cambridge, UK.

Key discovery

Chemical Transmission of Nerve Impulses: A Historical Sketch. By Z. M. Bacq. Pp. 106. (Pergamon: Oxford and New York, 1975.) £6 net.

THIS translation of Professor Bacq's book was distributed by the publishers free of charge (in a paper-bound version) to 500 participants at the Dale Centennial Symposium of the Physiological Society in 1975. It is a pity that the hardback volume now published is so expensive and that the paperback version has not been made generally available, because the book is a delight to read and is more important than its small size implies. The discovery of the chemical transmission of nervous impulses must surely rank as one of the major discoveries in the biological sciences and so a brief account of the experiments and personalities behind the discovery is very welcome. Professor Bacq was himself involved in some of the early work with Cannon, Fredericq and Dale, and so his book is of especial value. He frankly admits that he is writing a personal view and I suspect that others who were involved in the research would not always agree with him. The virtue of this slightly subjective view of history, however, is that it involves the reader in the spirit of the time and gives him a real feeling for the excitement of discovery.

The first two very short chapters introduce the topic for the non-biologist; then we are told a little about the early workers who clearly expressed the hypothesis of chemical transmission but were unable to do experiments that satisfied the sceptics of the time.

In his description of the discovery of cholinergic transmission, the author raises a few doubts about some aspects of the autobiographical sketch which Loewi wrote in 1960 (at the age of 80). Bacq goes on to state: "The great merit of this exceptional man [Loewi] was his ability to follow a logical course and to accumulate convincing experimental evidence without paying too much attention to the criticisms of his theory". A few of Feldberg's beautiful experiments are then described, which are as convincing as any of Loewi's and which were carried out on the living animal.

Credit for the first experiment which showed that a chemical substance is involved in transmission of impulses from one nerve to another nerve (in the superior cervical ganglion) is given to Kibjakow (1933). Kibjakow's elegant experiment did not, however, throw any light on the nature of the substance released from the preganglionic fibre; it was Feldberg and Gaddum

who proved that it is acetylcholine. Bacq's account of transmission to striated muscle is strangely incomplete, for he does not mention the critical experiments of Dale, Feldberg and Vogt (1936) in which they identified acetylcholine in the perfusates from several voluntary muscles after stimulation of the motor nerves.

The account of the author's own experiments with Cannon on adrenergic transmission is not only fascinating but serves to correct those who judge Cannon's work only by his entanglement in the erroneous hypothesis (attributed by Bacq to Rosenbluth) concerning 'sympathin E and I'.

Perhaps the most amazing story in the book is to be found in the chapter describing the opposition by Eccles and Heymans to the idea of chemical transmission. The reader becomes a little sceptical about Eccles' use of Popper's philosophy to 'explain' his reluctance to accept sound experimental evidence over almost a decade.

Bacq's book should be read by all students of physiology, especially those intending to do research in the neurosciences. It is not, and does not pretend to be, a complete and objective history of the subject. One can only hope that this vivid account will provoke others who participated in the research to write down their recollections. Then, one day, the full story of this key discovery of modern biology will perhaps be written.

A. D. Smith

Dr Smith is a Lecturer in Pharmacology at the University of Oxford, UK.

Classic physics

Three Phases of Matter By Alan J. Walton. Pp. xiii+492. (McGraw-Hill: London and New York, June 1976.) Hardcover £15; softcover £5.95.

THIS text is suitable for students embarking on science courses with a major or minor element of physics at University (or equivalent) level. It had its origins at Sussex where—I must here declare my interest—I worked briefly with the author in a lecturing double-act. Many of the arguments developed in the book had already taken shape then (1969) but the experience of the Open University, with its disciplines of clarity of presentation, has had a strong and beneficial effect.

The subject matter is the classic, if recently less fashionable, one of solids, liquids and gases. The style (quasi-conversational he calls it) is inimitable Walton. It is used to convey the impression that the author stands outside the 'establishment' as a sort of wise uncle always trying to bring an argu-

ment down to earth by relating it to everyday experience. "As is often the case," he says, "one can learn much from analogues." In fact the dependence on analogues and other physical models is very great.

Much basic groundwork is covered in the first three chapters (the p - V - T surface, characterising atoms, temperature) before the three phases (three chapters on gases, four on solids, two on liquids) are discussed. Very little previous physical knowledge is assumed or needed and the mathematics is kept to a minimum, although even that minimum is in places demanding as, for example, in the one-dimensional coupled oscillator model of a solid. There are liberal supplies of exercises and problems, with comments and/or answers.

The extreme care with which physical ideas are usually introduced and used is a feature of the presentation. I can't say that I would always agree with the author, and I feel that most readers will find parts which they find unnecessarily idiosyncratic and perhaps condescending. I read the book from cover to cover and I liked it. I hope there is a market for it.

D. S. Betts

Dr Betts is a Lecturer in Experimental Physics at the University of Sussex, UK.

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Nucleic acids revised

The Biochemistry of the Nucleic Acids. Eighth edition. By J. N. Davidson. Pp. xii+420. (Chapman and Hall: London, October 1976.) Hardcover £8.50; paperback £4.50.

THE late Professor Davidson wrote seven invaluable editions of this book over twenty years of exciting discovery in nucleic acid biochemistry. The eighth edition by four of his former students and colleagues at Glasgow (R. L. P. Adams, R. H. Burdon, A. M. Campbell and R. M. S. Smellie) packs a mine of information into a volume that can still be put into a coat pocket. It emphasises the enzymology of nucleic acids with a sound foundation of their structural analysis and physico-chemical properties. Some chapters—especially that on the replication of DNA—form really advanced reviews. An excellent feature is the skill and authority with which important properties of prokaryotic and eukaryotic polymerases are compared. The book is copiously documented with many important references, has few errors or ambiguities in proportion to the wealth of its content, and is priced very reasonably, especially in the paperback edition.

Although this new edition will follow its predecessors in being an important source book in nucleic acid biochemistry, the role of the book may perhaps be changing. Nucleic acids were, for many years, treated poorly in even the best general biochemistry texts. Today, such texts may contain excellent sections on nucleic acids and Davidson's book is no longer needed to introduce students to the field. It still has no equal in providing the necessary background for those who are contemplating or beginning a research project in nucleic acid biochemistry.

Yet it is a pity that the eighth edition has lost the freshness of the first and does not bubble with the excitement and imagination that is still typical of much nucleic acid research. Some sections—for example, those on DNA sequencing or protein synthesis, are quite disjointed, presumably as a result of too much scissors and paste. There is no glimmer of the rich prospects opened up by the ability to recombine DNA *in vitro* and to clone the artificial recombinants. Several good electron micrographs of nucleic acids are shown, but the techniques are not outlined, and the value of electron microscopy is not emphasised enough. The relationships between nucleus and cytoplasm as may, for example, be illustrated in histone biosynthesis are

not explained. The relevance of 6-O-alkylation of guanine to chemical mutagenesis is not mentioned. Indeed, mutagenic screening tests to identify potential environmental carcinogens are not discussed.

In view of its reasonable price, one can forgive most of the cramped black-and-white diagrams. But some are so bad they cannot be excused. In particular, the numbered purine and pyrimidine ring structures are so tiny that they can hardly be read. Certain diagrams of replicating *Escherichia coli* DNA do not show two-directional replication and one shows linear chromosomes. A bacterial cell is drawn to imply that all ribosomes occur free in the cytoplasm instead of being generally attached to messenger RNA which can still be attached to the DNA.

Despite the usefulness of this new edition and the extensive revision that it has undergone, I think that more revision is still needed as the pace of discovery has been fast. Dubious or outdated items should be pruned, certain diagrams revised and the authors encouraged to bring out crucial features of selected experiments and to re-introduce a forward look.

Kenneth Burton

Kenneth Burton is Professor of Biochemistry at the University of Newcastle upon Tyne, UK.

Perspective on penicillin

Penicillin in Perspective. By David Wilson. Pp. xi+298. (Faber and Faber: London, October, 1976.) £4.95.

It is now nearly half a century since Fleming's famous observation of the action of penicillin on a contaminated plate. This was followed, over a decade later, by the demonstration of its chemotherapeutic possibilities by a small group of workers at Oxford led by the late Lord Florey, which revolutionised the treatment of infection and triggered the explosive development of the multi-million pound antibiotics industry.

Because of its glamour and the ease with which the early work on penicillin can be grasped by the layman, several popular books on it or on antibiotics in general have appeared. Can yet another be justified? On reading the most aptly named *Penicillin in Perspective*, initial scepticism on this point is quickly replaced by respect for the thoroughness with which the author has researched this material and the

freshness of his approach; even those already familiar with the story will find more than a little that is new to them. The interweaving of the main theme with relevant background is skillfully done, and there are fascinating side-lights on personalities and circumstances. The part played by luck repeatedly recurs, and among a number of interesting speculations is whether, in these days of stringent official control of new drugs (perhaps partly in response to ill-informed public clamour) penicillin would ever have reached clinical medicine. There are a good number of quotations, some quite long, from original papers, from correspondence and from other books, which add interest and authority, and complement the author's compact but clear style.

It is good to find credit given to some of the little-known workers in the field, such as F. Ridley and (the late) Stuart Craddock, two medical students at St Mary's Hospital who, working under great difficulties, prepared a protein-free penicillin which could surely have been used for protective experiments in mice. Precisely why these crucial experiments were not attempted will never be known, but a fairly convincing explanation is offered in terms of the general scientific outlook at the time, the particular climate of opinion in Wright's laboratory, and Fleming's own character.

Few significant errors have been noticed: the barium salt of penicillin was not crystallised in 1941 (p183); penicillins I, II, III, and IV in the British nomenclature correspond to F, G, X and K respectively in that of the US (p16). A more serious inaccuracy is the claim (by the author) that the Oxford workers "found out how to manufacture penicillin on a commercial scale" (p141). It is true that they carried out culture of the fungus on what, for a research laboratory, was a very large scale, but the yield of penicillin was so painfully low (at most 2 units ml⁻¹) that the total production to the middle of 1941 and the completion of the trials on the first six parenterally treated patients at the Radcliffe Infirmary, was probably less than 5 megaunits. With modern high-yielding strains of the fungus in deep culture this amount could be obtained from less than half a pint of culture fluid!

This is indeed a worthwhile book and presents a sober, critical and well informed view of penicillin in perspective.

N. G. Heatley

N. G. Heatley was formerly Senior Research Officer, and is now Lecturer, at the Sir William Dunn School of Pathology, University of Oxford, UK.

announcements

Appointments

P. H. Parkin, of the Building Research Establishment, to be Visiting Professor and **J. S. Ellis**, formerly Professor of Orthopaedic and Accident Surgery, to be Emeritus Professor, both in the University of Southampton. **D. Phillips**, Senior Lecturer to be Reader in Chemistry, University of Southampton.

Professor Friedrich Deinhardt to the Chair of Hygiene and Medical Microbiology, and Director, Max v. Pettenkofer Institute, Ludwig-Maximilians University of Munich, from March 1, 1977.

The Clinical Research Centre at Northwick Park, Harrow has established three new divisions of Immunological Genetics, Perinatal Medicine and Rheumatology. The heads of these divisions are, respectively, **Winfred Watkins**, **F. E. Hytten** and **Barbara Ansell**.

Meetings

January 7, 1977, **The Drought of 1975-6**, London (Royal Meteorological Society, Grenville Place, Bracknell, Berkshire).

February 17, 1977, **Europe—the Directive and the Cosmetic Industry**, London (Society of Cosmetic Chemists, 56 Kingsway, London, WC2).

March 7-11, **Stability of Tropical Environments and Populations**, Panama (Dr R. T. de Araúz, P.O. Box 662, Panama 1).

March 31-April 1, **Semiconductor injection lasers**, Cardiff (Institute of Physics, 47 Belgrave Square, London, SW1).

April 18-22, **Molecular Beams**, Noordwijkerhout, Holland (Louise Roos, FOM-Institute for Atomic and Molecular Physics, Kruislaan 407, Amsterdam).

May 2-6, **Protides of the Biological Fluids**, Brugge, Belgium (Jerusalemstraat 34, B-8000 Brugge).

May 30-June 3, **American Geophysical Union**, Washington (AGU, 1909 K Street, N.W., Washington, DC 20006).

June 15-24, **Advanced Institute in Methods of Immunological Diagnosis**, Bloomfield Hills, Michigan (N. R. Rose, Wayne State University School of Medicine, 540 East Canfield, Detroit, Michigan, 48201).

Person to Person

Reading-Paris. Accommodation exchange for six months (approx.) from January/February 1977. We offer 3 large bedrooms, fitted kitchen, full central heating, garage and use of vehicle, if required, plus four other rooms. Quiet rural area, large garden. House is near to NIRD, Shinfield with easy access to Reading University. Anything in Paris considered for self and wife, preferably accessible to 16th Arrond. Write to c/o Wilkins, Spencers Wood Post Office, Reading, Berkshire, UK.

Frank Horne Memorial. A fund is being established to commemorate the former director of the National Institute of Agricultural Botany. Grants will be awarded from the fund for education and research in the production of seeds, plants and crops. Contributions and enquiries to The Secretary, NIAB, Huntingdon Road, Cambridge.

Adenia digitata Engl. Investigator would like to know a reliable source from which to obtain seeds, fruits and roots of this plant. Please write to Professor F. Stirpe, Istituto di Patologia generale, Via S. Giacomo 14, 40126 Bologna, Italy.

Furnished 4 bedroom house in Winchester, Massachusetts offered in exchange for 2-3 bedroom accommodation in Cambridge, England for four months, May-August, 1977. Winchester is within easy commuting distance of Harvard, M.I.T. and all major hospitals in Boston, and has an excellent school system. Dr Ajit Kumar, Shriners Burns Institute, 51 Blossom Street, Boston, Mass. 02114.

There will be no charge for this service. Send items (not more than 60 words) to Marcus Dobba at the London Office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

June 18-19, **Gut Hormones**, Lausanne, Switzerland (S. R. Bloom, Department of Medicine, Hammersmith Hospital, Du Cane Road, London, W12).

June 27-30, **Genetics Society of Canada**, Winnipeg (Phyllis McAlpine, Department of Genetics, 685 Bannatyne Avenue, Winnipeg, Manitoba).

July 4-6, **Rare Earths and Actinides**, Durham (Institute of Physics, 47 Belgrave Square, London, SW1).

July 10-22, **Sensory Ecology (NATO Institute)**, Lennoxville, Quebec (M. A. Ali, Département de Biologie, Université de Montréal, C.P. 6128, Montréal, Canada).

August 2-5, 1977, **Cryogenic Engineering**, Boulder (Dee Belsher, National Bureau of Standards, Boulder, Colorado, 80302).

September 12-15, **Solid State Devices (ESSDERC 1977)**, Brighton (Institute of Physics, 47 Belgrave Square, London, SW1).

September 26-29, **Phase Transition in Bulk Polymers**, Varna, Bulgaria (M. Mihailov, Central Laboratory for Polymers, 1113 Sofia, Bulgaria).

Reports and publications

Great Britain—October

Waste Management Advisory Council. Paper No. 2: Report on Waste Paper Collection by Local Authorities. Pp. vi + 25. (London: HMSO, 1976.) 60p. net. [410]

Scholarships Guide for Commonwealth Postgraduate Students, 1977/79. Pp. 346. £3.75. Some Awards Open to Graduates of Foreign (non-Commonwealth) Universities and Tenable at UK Universities. Pp. 48. £1; \$3. (London: The Association of Commonwealth Universities, 1976.) [610]

Memoirs of the Royal Astronomical Society. Vol. 82, Part 2: Studies of A and F Stars in the Region of the North Galactic Pole—I. By G. Hill, A. Allison, W. A. Fisher, G. J. Odgers, E. L. Pfennersmidt, P. F. Younger and R. W. Hilditch. Studies of A and F Stars in the Region of the North Galactic Pole—II. By R. W. Hilditch, G. Hill and J. V. G. Barnes. Pp. 69-94. (Oxford and London: Blackwell Scientific Publications, 1976. Published for The Royal Astronomical Society.) [710]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 282, No. 1311: A Study of Unsteady Forces at Low Reynolds Number—a Strong Interaction Theory for the Coaxial Settling of Three or More Spheres. By S. Leichtberg, S. Weinbaum, R. Pfeffer and N. J. Gluckman. Pp. 585-613. (London: The Royal Society, 1976.) UK £1.50, Overseas £1.60. [810]

The Royal Scottish Museum. The Scottish Society of the History of Medicine. The Early Years of the Edinburgh Medical School. Edited by R. G. W. Anderson and A. D. C. Simpson. Pp. 124. £3 net. Edinburgh and Medicine. Compiled by R. G. W. Anderson and A. D. C. Simpson. Pp. 72. £1.75 net. (Edinburgh: The Royal Scottish Museum, 1976.) [1110]

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nature

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Reprocessing: right to pause and think

HISTORY will almost surely record that the 1970s were years when the industrialised world had more than one occasion to pause and think about where it was going, and where it was taking everyone else. With the over-riding disposition still to 'go for growth' and environmental issues and energy problems assuming greater importance, it doesn't take a wise man to spot that just such an opportunity is now with us. Fortunately it looks as though the west's leading powers think so too.

The issue that prompts a pause is the reprocessing of nuclear fuel. As the following articles indicate, the question of whether to push ahead with this activity still further is now under scrutiny in Britain and the United States. A US moratorium on reprocessing and the export of reprocessing technology, called for by President Ford in October, has been followed by a similar decision from France last week. And an announcement is expected from Canada before the end of the year.

As with all international issues, these developments have aroused some suspicion about the motives behind them; not every country, after all, is at this moment equally well placed to reprocess nuclear fuel. But if not too much ought to be made of such events, it is nevertheless right that they have occurred. The three contemporary concerns of growth, energy and the environment are now acting to reinforce each other in the public arena, and the focus they have found is nuclear power.

At the heart of it all is the question of whether a fast breeder reactor system should be developed, but that question, though important, is receiving somewhat disproportionate attention. This may be because of a misguided belief that a decision on the breeder is a decision on the future of reprocessing. If the breeder doesn't receive the green light it does not automatically follow that reprocessing fuel from thermal reactors as a

commercial operation has no purpose. What is often forgotten is that plutonium, the retrieval of which is the principal object of reprocessing (though obviously the uranium obtained is useful too), can also be used in oxide-fuelled thermal reactors. That means a decision on reprocessing, the most radioactive stage in the nuclear fuel cycle, is quite as important as a decision on the breeder.

To see better why this is so needs some elaboration. Reactors in service now use either natural uranium metal fuel (Magnox), natural uranium oxide (CANDU) or slightly enriched uranium oxide (AGR and PWR). Spent fuel taken from Magnox reactors and put to 'cool' under water can, after a period that ought not to exceed about a year because of corrosion, either be reprocessed to yield practically all the uranium and plutonium, together with high level waste which remains active for hundreds of years, or dealt with directly as high level waste which remains active for thousands of years.

The waste after reprocessing is at the moment kept in enormous stainless steel tanks. Techniques to render it safer and easier to handle through a process of glassification are under development, and reliable sites for its disposal are also being sought. The problems are essentially the same whether reprocessing is done or not. The increase in volume of waste that has to be handled is obviously considerable if reprocessing is not done, and the much longer period for which un-reprocessed fuel remains active means that prospective sites must be correspondingly more reliable geologically: but these differences are almost marginal at this stage, and offer only a rather perverse case in favour of reprocessing.

In practice there is little realistic, or economic, option but to reprocess the Magnox fuel. The engineering problems associated with the vitrification technique have yet to be ironed out, and as the spent fuel elements build up in the

cooling ponds there is a greater danger in not reprocessing as long as alternative ways of keeping the spent fuel are unavailable. Both Britain and France are reprocessing Magnox fuel on a commercial scale to retrieve uranium and plutonium. To that extent they are already in the so-called 'plutonium economy', which so many people wrongly believe is something which only comes in with the fast breeder.

The spent fuel of both CANDU reactors and of AGRs and PWRs, like that of Magnox-fuelled reactors, contains plutonium and uranium; uranium from AGR and PWR fuel is richer in uranium-235 than natural uranium, and this gives it extra value. What makes the question of oxide fuel reprocessing different from any question associated with Magnox fuel reprocessing is that the need to reprocess oxide fuel is not urgent. Indeed, with the right precautions, spent oxide fuel can be stored under water for a matter of years if not decades without significant danger, although those arrangements obviously should not be permanent and would create surveillance problems.

No commercial-scale oxide reprocessing plant is yet in operation, and on present calculations none is expected to begin before the 1980s. But the case for having one is now being pressed heavily by the nuclear industry. At one level the arguments make the point that the hazards attached to the process are not greater than with the already-accepted Magnox fuel reprocessing, save that oxide fuel is substantially more radioactive and produces more waste afterwards. Important as that is, as a matter of industrial danger the point is separate from the worries about plutonium, which are not answered simply by the fact of what is, after all, an imposed choice regarding the reprocessing of Magnox fuel.

The case for oxide fuel reprocessing is anyway being made at another level. It turns on the already-mentioned fact that plutonium does not just have military purposes when it is not used to

fuel breeder reactors. Oxide-fuelled thermal reactors can be adapted to take a mixed oxide (plutonium oxide-uranium oxide) fuel. Whereas the breeder uses 20% plutonium oxide, though, the adapted thermal reactors would use something closer to 2-3% plutonium oxide.

The plutonium would be used less efficiently than in a breeder, but this 'plutonium recycle', together with enrichment of the retrieved uranium (an operation presenting no new hazards), would clearly extend the life of available nuclear fuel. To reprocess, in other words, is to lengthen the period for which the world has nuclear power, quite regardless of the fast breeder, at a time of burgeoning uranium prices and limited uranium supplies.

It is a nice argument, because it emphasises both economy and resource use. But the additional period for which the world would have nuclear power is disputed. It is certainly not long by comparison with the supposedly limitless extension that a fast breeder system would allow. Indeed, the uranium saved may not be at all great given the massive fuel-consuming reactor programmes envisaged for the future; and the money saved will, in many people's estimates, be quite marginal by comparison with the huge capital costs involved in generating nuclear power—costs which won't decline while engineering problems, licensing requirements and environmental regulations grow.

If the broad economic case for reprocessing looks unproven that may be because too much depends on what price people are willing to pay. But that in turn means assessing what risks people are prepared to take. The same is true of the arguments about resource use, which raise the very questions of moral responsibility that environmentalists who campaign against oxide fuel reprocessing would urge be taken into account. The industry says it is irresponsible not to prepare for the fast breeder option if and when it comes, and not to use plutonium as a fuel in thermal reactors if not breeders. The response it receives is that people must first decide, on the basis of an assessment of risks involved.

There is evidence that this is now happening. Certainly many of the worries about plutonium have been well publicised. The industry acknowledges the problems but says they are not insuperable and ought not to worry people as much as they do. There is indeed a need for greater perspective—the risks associated with overeating, drinking, smoking and driving, and from existing toxic substances, are probably greater. But when it comes to proliferation, it is another matter.

The industry says the safest place for retrieved plutonium is in a reactor, and sees the move to oxide reprocessing as a change of degree rather than kind. But it is precisely the change in scale that is worrying many people, since there is hardly likely to be a greater insurance against danger with the wider use of larger amounts of plutonium. The serious aspect of reprocessing, though, concerns the international trade in the technology, for this is the nub of the proliferation problem. The capacity of any country to produce nuclear weapons is eased considerably if it has its own reprocessing plant.

Among the countries now operating or building experimental or pilot-scale plants are West Germany, Japan, India, Italy, Spain, Yugoslavia, Argentina and Taiwan. Brazil is expecting a plant from Germany, and Pakistan is expecting one from France. Whatever the fears about the use by terrorists of plutonium, the fact remains that it is governments which have made and used bombs in the past, and governments which are likely to make and use them in future. Governments are sovereign to sufficient degree that even the best safeguards can be flouted that depend finally on faith for their effectiveness. Today's government may in any case be tomorrow's guerrillas.

The discussions of the Nuclear Suppliers Group—the exporters of nuclear technology—are thus too important to take place in the secrecy that has beclouded them so far. The lack of information on these talks has helped to prevent the discussion of the issues which is now necessary. But even if the group manages to agree to abandon exports of reprocessing technology, it must acknowledge that other countries have nuclear programmes, and will then have to decide whether reprocessing, if it is to be done at all, is to be done at an international reprocessing centre.

The industry's argument, that proliferation is here to stay anyway, is strong. There is, it says, enough published on the subject of reprocessing for a determined nation with a nuclear reactor to have a small-scale plant secretly (if inefficiently) helping to make weapons, at not too prohibitive a cost. There are also probably enough people in the business who know about the subject to assist in such a cause. And who would dare stand up and say that other countries should not have nuclear power in the first place?

If this is unanswerable, other questions do need a proper response before anyone concludes that an international reprocessing centre is the best solution. Will the creation of such a centre remove the incentive for others to re-

process altogether, or because of its attractions (and the precedent) encourage others to do it too? And will it induce other countries justly seeking energy independence to go nuclear over-hastily, or deter them completely?

And the prior question of whether there should be reprocessing at all must be answered first. A decision against reprocessing, if sustained, would be a decision against a nuclear future, and to that extent against nuclear power. But at least it would be a reversible decision which would not later preclude lengthening the resulting nuclear interlude finitely (by allowing reprocessing to go ahead) or almost infinitely (by allowing a system of fast breeders). A decision the other way permits no such flexibility.

Given the world's energy problems, though, there is a clear need for early decisions to be made at the highest levels on the subject of reprocessing. The powerful multinational nuclear industry, of course, though it has grown encouragingly more sensitive to public opinion, wants quick decisions because of the long lead-times on its investments and because it wants to secure the exports it needs to survive. But these should still only be made after the fullest possible informed debate before the widest possible public, a requirement that has not yet been fulfilled. This is the increasingly salient 'legitimacy factor' in the nuclear business; it demands that consent be mobilised through the usual channels of public debate, be they letters to local papers or national referenda.

A decision not to reprocess at all would imply a particular view about world energy which the industry cannot accept. For the vast majority of the world's population, that view may not be as gloomy as it has been painted. Their needs, it is often said, would be served by eschewing nuclear energy in favour of natural sources appropriate to a course of economic development that does not duplicate the course already taken by the industrialised world.

Perhaps in this perspective, which comes from those concerned at the widening gap between developed and underdeveloped nations, there is something for the industrialised world to learn. After all, if the energy crisis is as serious as people say, it ought to be tackled seriously, and not simply with proposals that unquestioningly offer more of the same. The reprocessing of oxide fuel is an issue which provides a suitable occasion for some of these matters to be raised. It may also be the single most important nuclear issue facing the world at this moment which is also amenable to political influence. The question is, is reprocessing worthwhile? It is at least right to pause and think. □

Reprocessing (1): Barnwell, USA

An inevitability forestalled

Colin Norman, recently at Barnwell, reports
on the reprocessing debate in the United States

AMONG the more controversial problems awaiting the attention of the Carter Administration next year will be whether to allow an extraordinary nuclear facility, built like a concrete fortress in the middle of a South Carolina pine forest, to start operating. Constructed by private industry at a cost so far of some \$250 million, the facility could become either a key component in the nuclear power programme in the United States, or a massively expensive white elephant.

Designed as the first full-scale commercial plant to reprocess spent nuclear fuel from power reactors, the facility is already falling victim to rising concerns about the proliferation of nuclear weapons, and its fate will probably hang on a complex series of regulatory, political and diplomatic decisions expected to be taken next year. But, even if the Carter Administration does decide to allow the plant to begin work, its troubles won't be entirely over. Before it can operate at full capacity, additional facilities to solidify highly radioactive wastes from the plant and to convert plutonium nitrate to plutonium oxide will be required. They could cost as much as \$500 million, a sum which private industry says it is unwilling to risk without government assistance.

When plans for the plant were drawn up in the 1960s, it looked like a sure-fire commercial proposition. Opposition to nuclear power had scarcely begun to surface, prospects for burgeoning growth in the nuclear industry looked good and reprocessing was generally regarded as inevitable. Three industrial concerns—the Allied Chemical Company (50%), Gulf Oil (25%) and Royal Dutch Shell (25%)—therefore decided to form a partnership, called Allied General Nuclear Services (AGNS), and sink about \$100 million into the venture. They expected to have the facility in operation by about 1974.

Enticing idea

The plant is designed to reprocess fuel continuously from about 50 commercial power reactors, extracting plutonium and uranium from highly radioactive spent fuel rods and recycling the materials as new reactor fuel. The idea looked enticing.

Light water reactors now in use in the United States and in most other

countries are fuelled with uranium in which the concentration of the fissile isotope uranium-235 has been enriched from its natural level of about 0.7% to about 3%. While the reactor is operating, small quantities of fissile plutonium are formed along with other highly radioactive products and when the fuel rods are removed from the reactor, they contain roughly 1% plutonium and 1% unused uranium-235—more than half the original amount of fissile material. If those elements could be extracted and recycled as reactor fuel, savings in fuel costs could be substantial, and the need to mine and enrich uranium would be greatly reduced.

Another incentive for commercial concerns to get into the reprocessing business was the expectation that large numbers of breeder reactors would be in operation by the end of the century. Since breeder reactors produce large quantities of plutonium, a plutonium extraction industry is essential to the economics of their operation. Small wonder, therefore, that Allied, Gulf and Shell decided to get into the reprocessing business.

They were not alone when they designed the plant in the 1960s. A small-scale reprocessing facility was already in operation at West Valley, New York, and another was on the drawing board for a site at Morris, Illinois. In addition, plutonium had been extracted from spent fuel from military reactors ever since the Manhattan Project in the early 1940s—a fact that gave would-be commercial reprocessors confidence that the process would work successfully. So far, however, the nascent reprocessing industry has been beset by disasters.

The West Valley plant, designed to handle about one ton of irradiated fuel per day, was operated from 1966 to 1972, when it was closed for modification and expansion. Since then, the estimated costs of modifying the plant have skyrocketed, uncertainties have arisen about how the wastes should be handled, and the amount of time required to complete the construction work has stretched to ten years. Consequently, the plant's operator, a consortium known as Nuclear Fuel Services, announced last year that the plant would be scrapped.

The second facility, constructed in the early 1970s by the General Electric Company at Morris, was an even

greater disaster. General Electric sank about \$64 million into the plant, but a number of problems with remote handling of radioactive material were encountered during testing and in 1974, the company decided to cut its losses and abandon the venture.

The AGNS facility is therefore the only plant remotely close to operating, though the Exxon Nuclear Company has applied for a licence to construct a similar-sized plant at Oak Ridge, Tennessee, in the mid-1980s. The facility, located near Barnwell, South Carolina, is generally acknowledged to be vastly superior technically to the West Valley and Morris plants; it is, however, facing a determined challenge from nuclear critics and arms control advocates, who argue that it would usher in a new and more dangerous nuclear age—the so-called plutonium economy. Opposition to the facility has, in fact, already caused long delays and is partly responsible for the cost escalation from the original estimate of \$100 million to the present cost of \$250 million.

Massive construction

Built to reprocess about 1,500 tons of irradiated fuel per year—the output from some 50 reactors—the facility is certainly impressive. A massive concrete construction, lined throughout with stainless steel, it is designed to operate by remote control. Irradiated fuel rods will be shipped to the plant in heavily shielded casks, and they will be stored in the facility in huge water pools. From there, they will be transferred to a giant hydraulic shearing device, which will chop the rods into two-inch sections, and the pieces will be plunged into a bath of hot, concentrated nitric acid.

The acid bath will dissolve uranium, plutonium and other radioactive elements from the fuel rods, leaving behind the stainless steel cladding, which will be removed and stored pending final burial. Plutonium and uranium will be removed from the nitric acid solution by a relatively straightforward solvent extraction process, and the plutonium and uranium salts will be separated from each other electrolytically. The waste solution will be concentrated and transferred to huge, double-walled stainless steel tanks for temporary storage.

A facility for converting uranyl nitrate from the separation plant to uranium hexafluoride has been built by AGNS at the Barnwell site, the idea being to ship the fluoride directly to an enrichment plant at Oak Ridge, Tennessee. As for the plutonium, the plan is to convert it eventually to plutonium oxide for fabrication into reactor fuel consisting of a mixture of plutonium oxide and enriched uranium

oxide. At present, however, no plutonium conversion facility has been built at Barnwell, and the element will therefore probably be stored temporarily as plutonium nitrate solution in titanium tanks.

Plutonium politics

A number of environmental and anti-nuclear groups, led by the Natural Resources Defense Council (NRDC), began to worry in the early 1970s about the consequences of pushing ahead with a programme to extract and recycle plutonium on a large scale. They argued that plutonium could be stolen from the nuclear industry by individuals or terrorist groups and fashioned into crude atomic weapons, and they also challenged the federal government's exposure standards for plutonium, arguing that they are much too lax. So far, they have achieved considerable success in their assaults on the programme.

In 1974, the Atomic Energy Commission, which was then responsible for licensing nuclear facilities, announced that it would publish an environmental impact statement on the potential consequences of a large scale plutonium recycling industry before deciding whether to give the Barnwell plant a licence to begin operating. The statement was published in August, 1974, and it recommended giving a green light to plutonium recycle.

A few months later, however, the Atomic Energy Commission was scrapped and its licensing functions were transferred to the Nuclear Regulatory Commission (NRC). The new regulatory environment hasn't helped the Barnwell plant. In January last year, the NRC announced that it would re-examine the AEC's impact statement, and publish an expanded version of its own. NRC also announced that it would subject the matter to an exhaustive public hearing before making any decision on reprocessing and recycling.

The first part of NRC's impact statement, dealing with health and safety questions, was published a few weeks ago, and the second part, covering the more sensitive issues of safeguards and cost-benefit analysis, is expected to be published early next year. The promised public hearings have begun, and they are expected to continue for most of next year. NRC officials say that they hope to reach a final decision on whether reprocessing and recycle should be allowed by January 1978, which means that even if Barnwell gets a green light, it would not be started up until 1979.

In the meantime, however, the regulatory process has been overtaken to some extent by political developments. The explosion by India of an atomic

device constructed from plutonium extracted from an imported nuclear reactor in 1974 set loose a major debate in the United States about the nation's responsibilities to try to curb the spread of nuclear weapons. Throughout the past year, US officials have been urging the adoption of strict international controls on nuclear exports and they have been arguing for a complete moratorium on the sale of reprocessing and uranium enrichment technology to nations which do not have nuclear weapons. Concerns about proliferation have, in turn, led to a re-examination of the United States' own plans for reprocessing, and the matter even played a prominent role in the Presidential election.

In a speech delivered at a United Nations meeting last May, President-elect Jimmy Carter announced that he had reservations about pressing ahead with plutonium recycle in the United States while the State Department is trying to persuade other nations not to follow the same path. In November, Carter was more specific. He said he would "seek to withhold authority for domestic commercial reprocessing until the need for, the economics, and the safety of this technology is clearly demonstrated. If we should ever decide to go forward with commercial reprocessing, it should be on a multinational basis". A few days before the election, President Ford came out with a similar statement. The nuclear industry in the United States should no longer assume that reprocessing will be "a necessary and inevitable step in the nuclear fuel cycle", he said, and he announced that he would permit reprocessing and plutonium recycle "only if they are found to be consistent with our international objectives".

Mixed reaction

Those statements were greeted with satisfaction by opponents of the Barnwell plant. Nuclear critics and arms control advocates had long been arguing that a decision by the United States to forego reprocessing, at least for the time being, would send a clear message abroad that the United States is serious about its non-proliferation objectives. Needless to say, the nuclear industry doesn't agree.

Dr J. A. Buckham, manager of the Barnwell plant, said in an interview last week that "almost all of the stated objections of opponents of reprocessing are best served by the very opposite of what they advocate". He argued that the best way to persuade non-nuclear countries against embarking on reprocessing is to ensure that reprocessing services are available for all, perhaps on a multinational basis. "The failure to start up this plant (Barnwell) will not persuade the rest of the world not

to reprocess", he said, and added that the plant is so well safeguarded that it would not add to the proliferation problem at all.

Buckham also noted that reprocessing and plutonium cycle would stretch out uranium reserves and to help reduce reliance on fossil fuels. In other words, it is a key to the long-term prospects of the nuclear industry, which is one reason why nuclear critics are so opposed to the operation.

It should be noted that neither Ford nor Carter entirely ruled out the possibility of operating the Barnwell plant. Carter suggested that it could be turned into a multinational reprocessing facility, and both argued that the economics and safety of reprocessing should be proven before embarking on a full-scale reprocessing industry. The nuclear industry argues that the best way to prove the technology would be to operate the Barnwell plant as a demonstration facility, with government assistance. That possibility was under intense consideration in the Ford Administration last year, and it hasn't been entirely ruled out.

Operation of the plant would, however, require considerable capital expenditure. At present, it has capacity to store plutonium nitrate from about 18 months' production. A plant to convert the material to plutonium oxide would therefore be required soon after the facility is put into operation. Similarly, there is only temporary storage capacity for about one year's worth of radioactive wastes, and a solidification plant is required. Those plants could cost as much as \$500 million.

In view of the uncertainties surrounding the future for reprocessing, AGNS is unwilling to make that large an investment, and it has asked the Energy Research and Development Administration (ERDA) to build the ancillary facilities. The company said that it would be prepared to buy the facilities from the federal government once they were licensed and the plant was in full operation. ERDA officials have indicated that though federal assistance would not be forthcoming immediately, the possibility has not been ruled out entirely.

The future for reprocessing in the United States is therefore uncertain. Nuclear critics, such as Arthur Tamplin of NRDC, are convinced that the Barnwell plant can be stopped, while AGNS officials predict that they will receive a favourable decision from NRC. The matter is likely to be decided eventually by Congress—particularly if federal assistance is required—and it should be noted that the climate for nuclear power there is likely to change next year with the pending demise of the Joint Committee on Atomic Energy. □

Reprocessing (2): Windscale, UK

'The β -in-air alarm is a yodel sound'

Gillian Boucher, recently at Windscale, reports on the reprocessing issue in Britain

PRESS reaction to the leak at the Windscale reprocessing plant in Cumbria have confirmed British Nuclear Fuels (BNFL) in its belief that the media are impossible. The recently reported leak of 100 gallons a day of weakly radioactive water from an old concrete silo containing solid wastes stored in water was discovered on October 10 and its source has still not been identified. But in spite of shrieks about contamination it seemed clear almost from the start that there was probably no danger to the health and safety of workers or anyone else.

There have been allegations of secrecy but the UK Health and Safety Executive is satisfied that BNFL carried out its only obligations in informing the Nuclear Installations Inspectorate after a few days (as the leak was not dangerous there was no obligation to report it immediately) and the unions at the beginning of November. Mr Anthony Wedgwood Benn, Secretary of State for Energy, to whom BNFL is responsible, learned of the leak on December 8 and informed the House of Commons the next day. He later gave the impression that BNFL had improperly withheld information, and is now insisting on receiving details of all future incidents as soon as they occur. But in fact it was the Health and Safety Executive, not BNFL, that was responsible for reporting accidents to Mr Benn, and its rule of thumb has hitherto been that if they are not dangerous he need not be told. BNFL may, therefore, have suffered more than it deserves.

The painful lesson BNFL may now be learning, however, is that it is bound to be accused of secrecy unless it willingly provides far more information than it is statutorily obliged to do. Unless it leaves as wide a margin in its obligation to inform as it does in its compliance with safety regulations the criticisms will continue unabated. In this instance BNFL would have spared itself some insults and probably not lost anything if it had informed Cumbria County Council of the leak, which was discovered while the council was considering BNFL's application for planning permission for a major expansion programme. BNFL's rather lame excuse was that the leak was such a clear indication of the need for new plant

that revealing it could be seen as twisting the council's arm.

Three decisions

Mr Benn's strong reaction is perhaps best explained by the three major nuclear decisions the government will soon have to make, or at least postpone, in the face of rapidly growing public concern. The first is whether to allow the expansion at Windscale that the Cumbria council has provisionally approved: refurbishing the Magnox fuel reprocessing plant, building a new oxide reprocessing plant and assorted research work including development of the vitrification of wastes. Mr Shore, Secretary of State for the Environment, may announce a decision on this this week. The second is whether to build the first commercial fast breeder reactor. Mr Benn is now saying that there is plenty of time to consider this and a decision may not be forthcoming for a year or two. The third is whether to continue with the commitment to use the British steam-generating heavy water reactor (SGHWR) for the next generation of nuclear reactors. At present Mr Benn seems likely to opt for another large coal-fired power station whose cost (£600 million) will preclude development of the SGHWR programme. The SGHWR programme is also threatened by the latest round of public spending cuts which will probably mean delays in nuclear development, and by the Central Policy Review Staff's analysis of the power plant manufacturing industry, released last week, which points out the poor export prospects for the SGHWR.

Mr Shore, having postponed a decision on the Windscale expansion, now has several choices. He can refer the matter back to the county council, call in the matter for his own decision—either positive or negative—or set up one of two types of planning inquiry. The first would consider only third parties affected by the proposals, that is, local people; the second, a Planning Inquiry Commission, would look beyond the purely local issues.

Strong pressure to say yes immediately has come from BNFL management and unions because of £600 million-worth of deals in the pipeline to reprocess oxide fuels from abroad which could be jeopardised by delays. According to

Con Allday, BNFL's Managing Director, prolonged delay would mean disaster. Mr Shore is under pressure from other departments including the Treasury and the Department of Industry not to forego the foreign earnings. Mr Benn in effect gave the green light last March when he permitted BNFL to seek overseas business and to continue negotiations with the Japanese over the reprocessing of their oxide fuel. The government has not authorised the use of resources in this connection, and Mr Benn's recent enthusiasm for answering parliamentary questions embarrassing to BNFL suggests that he has misgivings. Mr Shore's inclination may yet be towards an inquiry, if only because it will be very difficult for him to call in any other planning application if he lets this one go. And the public hostility to nuclear power that has been fanned by the news of the leak is bound to influence his thinking.

Growing controversy

A year ago a decision not in BNFL's favour seemed inconceivable; nuclear power was not the issue in Britain that it was in the United States. Though the questions have remained essentially the same the number of people talking about them has greatly increased; certainly the opposition has had a better press than BNFL. And a shadow of concern on the part of such a body as the Flowers Commission perhaps changes more minds than the clamouring of half a dozen pressure groups.

BNFL insists that reprocessing is necessary for waste management, but for oxide fuels, which can be stored for decades, the requirement is not an urgent one; if the plutonium were not to be used—the controversial question—one eventual possibility might be to reduce the bulk of the spent fuel by removing the uranium and to vitrify the plutonium together with the wastes. Aside from plutonium, it is profits that make BNFL interested in reprocessing now.

The proposed expansion will cost £600 million of which half will be for the oxide plant; but a large part of the capital costs would be met by future customers for oxide reprocessing. The rest is expected to come from retained profits and loans from the private sector; £100 million was raised recently from a consortium of British and foreign banks. As the fuel from the British oxide reactor programme will only occupy 20–30% of the oxide plant's capacity there is plenty of room for those desirable export orders. An important political consideration is that expansion would provide another 1,000 jobs over the next five years to depressed Cumbria. And delays might

cause all this to be lost, possibly to the French or to the Americans, whose moratorium on foreign reprocessing extends only for three years.

But some questions remain to which it is difficult to get a clear answer. BNFL is a partner in United Reprocessors, an Anglo-French-German market-sharing body; just as the French came in on what was originally an Anglo-Japanese deal, it has not been explained what would prevent the British from demanding a share at any time in a Japanese-French deal even if there were a few months' delay for an inquiry. There would be a clause allowing the Japanese fuel to be returned un-reprocessed if the vitrification process were not developed satisfactorily, but a hefty downpayment that had meanwhile been invested in plant would be a lot to ask the British taxpayer to return to Japan. And though the job issue was until recently the one that mattered most in Cumbria, there are suggestions that the latest leak has swung many locals in favour of caution and an inquiry.

The other reason for reprocessing—the development of the fast breeder reactor which will use plutonium fuel—is itself in doubt. Even if the next stage, the CFR-1, goes through, Mr Benn's advisers are taking seriously the possibility that electricity demand will remain pretty much at present levels for the next 20 years and large capital expenditure on power stations around the year 2000 will not be necessary. There is already enough plutonium to fuel one commercial-scale fast reactor, and there are obvious disadvantages to separating out a lot more.

Safety issues

If reprocessing makes commercial sense or is necessary because of the fast breeder programme—both of which are of course hotly contested—there remain the issues of the safety of reprocessing and all that the plutonium economy entails. As far as the safety of reprocessing as an industrial process is concerned, the recent leaks look like supporting the Flowers Commission's remark that BNFL's housekeeping is not all it might be. But BNFL is careful to keep workers' radiation doses a long way within permitted limits, the yodel sound of an alarm is of course but one of an elaborate system of warnings and safeguards. So far nobody has been shown to have died as a result of his work at Windscale (the unions are at present seeking compensation for the families of three workers who have died of various cancers but BNFL is contesting the claim)—a record that few other large industries could claim. Suggestions have been published, however, that a succession of minor accidents with very similar causes shows that the

safety engineers are not learning as much from their past mistakes as BNFL claims.

Radiation levels in the Celtic Sea have caused a good deal of concern. The latest government figures show that the amount of alpha radiation is well within permitted limits but concentrations of beta emitters have risen steeply to reach a worrying 83% of permitted levels by 1975. The most dramatic increase—the tenfold rise in caesium levels in fish between 1973 and 1975—is mainly due to the corrosion of Magnox fuel elements stored for too long before reprocessing and releasing caesium into the cooling water; that should be corrected by the proposed improvements to the Magnox plant. But to increase throughput to the extent envisaged without increasing the dumping of radioactive elements in the sea will surely challenge BNFL's engineers.

Reprocessing oxide fuel is undoubtedly going to be a difficult business with very much higher levels of fission products and plutonium to deal with. Critics point to the 1973 accident at the experimental 'head-end' plant for pretreating oxide fuel which contaminated 35 men, but too much should not be deduced from what happened in a somewhat makeshift extension of the Magnox system; BNFL repeatedly point out that you cannot expect it to achieve the highest safety standards if it is not allowed to invest in new, purpose-made equipment. Many feel that if oxide fuel is to be reprocessed anywhere in the world, Windscale is probably one of the best places to do it.

Other problems, such as the storage of waste and even the protection of plutonium from terrorists, have a large technological component and could probably be solved given time and money. BNFL has suggested that plutonium fuel for fast reactors could for example be mixed with uranium oxide and poisoned with a gamma emitter to make it as unattractive a proposition for a criminal as the spent fuel which is transported unguarded from power stations to Windscale. Plants could be designed to make pure plutonium inaccessible. The unsavoury accompaniment of the plutonium economy, the big-brother surveillance, seems less of an issue in the good-humoured atmosphere of Windscale, where half the workers are locals and twenty-five years' service is not uncommon.

Critics emphasise proliferation

Friends of the Earth, the most persistent and articulate critics of reprocessing, would not accept that these problems are easily soluble, but their main point of attack is over nuclear

proliferation. BNFL complain that Friends of the Earth are always changing their ground: not long ago they attacked nuclear power plants, then they shifted to reprocessing and now they say that Magnox reprocessing is acceptable but oxide reprocessing should be prevented. Friends of the Earth reply that they learn as they go along and "try to find issues with a cutting edge within the general policy framework"—which is pro-conservation and small-scale energy production, rather than specifically anti-nuclear.

On the same side as Friends of the Earth are the myriad other pressure groups and individuals—the Conservation Society, the Lawyers' Ecology Group, Half Life, assorted academics and bishops. Ranged against them, at least in theory, is the establishment: BNFL, their owners the Atomic Energy Authority, the nuclear contractors the National Nuclear Corporation, the Central Electricity Generating Board, even some government departments. Additionally and interestingly, the trade unions have been solidly in favour of reprocessing and expansion. In practice BNFL, being the dirty end of the business, gets most of the criticism and has to put up most of the defence. In between, and supposedly helping in the nuclear debate that Mr Benn favours, are the media, many of which have been a good deal more interested in alarming than informing the public.

Both sides claim the Flowers Commission is with them and those who are puzzled by the delicate path trodden by Sir Brian Flowers suggest that he has changed his mind on reprocessing. Perhaps, as Flowers has said, the difficulty lies in the fact that "beginning with an optimistic view of the science and technology of nuclear power in the normal course of events, [the Commission] ended in such a cautionary fashion towards establishing too quickly the major reliance upon nuclear power that the development of commercial fast breeder reactors is intended to facilitate".

There are certainly plenty of questions to which a planning inquiry could address itself, among them the consequences to Windscale of stricter limits on air and water pollution, the reasons for the difficulties that virtually every oxide reprocessing plant in the world has run into, the feasibility of glassification, the question of design to withstand sabotage and the economics of reprocessing. A Planning Inquiry Commission might also consider the disposal of plutonium. Most of these issues were touched upon by the Flowers Commission but not in great detail. The mere fact that such a list of grey areas can so easily be compiled is now seen as perhaps the strongest argument for an inquiry. □

Radiating doubt

Australia's parliamentary debate on the Fox Report ended earlier this month. **Peter Pockley** reports from Sydney

IF anyone ever needed confirmation that uranium "debates" essentially reflect the politics of power and not the supposedly cool rationality and ultimate certainty of the scientific approach, the current Australian situation provides a nice case study. To the protests of the environmental movement, the Fraser government has announced a decision to allow mining to proceed on some uranium leases to fulfil existing export contracts with West Germany, Japan and United States for a total of 10,700 tonnes of uranium oxide.

This decision was announced within two weeks of the release of the long-awaited *First Report of the Ranger Uranium Environmental Inquiry** under the chairmanship of Mr Justice Russell Fox (hence the popular description "The Fox Report"). Fifteen months of thorough and judiciously fair collection of evidence and viewpoint, and detailed analysis thereof, was concentrated into the report which was commissioned in July 1975 under the Whitlam government's Environment Protection (Impact of Proposals) Act (1974). Three men—Judge Fox, medical Professor Charles Kerr and engineer Graeme Kelleher—were charged with examining a proposal by Ranger Uranium Mining Pty Ltd to recover 85,000 tonnes of U_3O_8 from an area 200 km east of Darwin in Australia's remote Northern Territory.

Because the total "reasonably assured and estimated additional uranium resources" of Australia amount, according to the Inquiry's figures, to about 353,000 tonnes of U_3O_8 —a significant fraction (nearly 10%) of the world total—the Inquiry accepted arguments that Australian decisions on uranium mining should be based on a thorough investigation of the nuclear scene world-wide. Indeed, when Mr Fraser tried to wind up the Inquiry by imposing an early deadline, as he was doing for most Whitlam-initiated inquiries which were in progress when he assumed office, Judge Fox publicly told him to go hang and Fraser had to give in. In retrospect, this was the first sign that there might not have been a close concurrence between the views of the Inquiry and the decisions of a government which is in favour of an expanding mining industry.

The First Report dealt with broad

issues brought to the fore by the discovery of massive uranium resources in several areas of Australia in the last 10 years, particularly in the Northern Territory. Though sparsely populated, much of the Territory is environmentally unique and also has large tracts of "reserves" through which the depressed Aboriginal peoples have special claims on discovered resources. The "findings" of the First Report are couched in generalised terms, with a sprinkling of—effectively—double negatives, which have allowed some differing interpretations by the original protagonists—miners and environmentalists—none of whom give the appearance of altering their original positions after the Report's publication. The Inquiry is now moving on to its next job, that of compiling a technical report on the environmental aspects of the proposed Ranger mine itself, and taking account of the untested effects of a recent Aboriginal Land Rights Bill.

To debate or not to debate?

The government's rapid decision to meet existing contracts, which had been put on ice pending the Fox Report, was clearly predetermined, and took scant regard of the Report's earnest final recommendation. This suggested

that no decision be taken in relation to the foregoing matters (i.e. the mining, milling and sale of uranium) until a reasonable time has elapsed and there has been an opportunity for the usual democratic processes to function, including, in this respect, parliamentary debate.

On announcing the government's decision, the Minister for Environment, Housing and Community Development, Mr Kevin Newman, promised a later debate in Parliament. The media divined that the issues at stake would split the country and both major political parties. The Labor Party and the union movement began to fulfil the prediction by publicly baring their souls, as has been their (destructive) wont in the past. In the event, however, the pragmatists in the Parliamentary Labor Party argued successfully in favour of retaining jobs for miners in the short term, and endorsed the government's green light for limited mining and export of uranium to meet existing contracts.

The increasingly confident uranium lobby—miners, the Atomic Energy Commission and the few nuclear experts in universities—engaged with

some aggression the numerically superior but collectively poorer environmentalists in strident clashes in the media, and the promised debate in Parliament, begun on the last day of November, was a fizz. Only a quarter of the total membership of the House of Representatives bothered to front up—or perhaps many members wanted to avoid being seen to be counted. The only highlight of the "debate" was the rare rebellion of one backbencher in the usually well-disciplined Liberal Party; Mr Don Chipp, a former minister passed over by Mr Fraser, proposed a two-year "moratorium" on uranium mining and export and threatened to cross the floor. The debate was defused, and probably buried, by being adjourned without a vote.

The environmentalists' hopes were then pinned on the union movement, elements of which had threatened withdrawal of labour on key links in the production chain (railway transport of processed ore to the ports, for example). But they were disappointed by the decision, early this month, of the powerful Australian Council of Trade Unions to endorse the government's decision. Thus has a test case, anticipated internationally as being of significance because of the thoroughness and (for uranium matters) rare openness of the Inquiry, become largely uninfluenced by public debate. The Parliamentary and industrial wings of the Labor Party have covered themselves by making strong noises against any extension of the present limited decisions to the sanctioning of uranium mining and export generally without "full public debate" or even a national referendum. But unless Labor wins power in Canberra again, it is virtually certain that the Fraser government will not stage such a debate and decision-making process.

Doubts remain

The Commissioners have become so worried about the government's interpretation of their final words that they are reported to have protested directly to Mr Newman saying that their two main conclusions are "findings" and do not amount to "recommendations" which can be used to justify the government's actions. The Commissioners must now be feeling that the wind of political forces may continue to blow so strongly over their heads as to drown out their voices.

The strongest thing the report seems to have still going for it is its uncompromising approach to the dangers of terrorist activity with fissile material and the inherent weakness of the Non-Proliferation Treaty and safeguards. They believe that proliferation is most likely to originate from fuel reprocessing plants and that "existing safe-

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guards may provide only an illusion of protection" On nuclear theft, the Report says "the risks are real and will tend to increase with the further spread of nuclear technology"

The moral argument against uranium mining and export, applied internationally, which says that Australia should not contribute further to the world's problems, has cut no ice with the government, which paints such protests as being against the national interest measured in terms of overseas earnings and jobs. In response, the environmentalists point to the Report's economic analysis which concluded that the contribution of the potential income from the Ranger deposit to national income is relatively small—only 0.1% in 1980–81, rising to about 0.5% in 1990–91, and declining to 0.4% by the end of the century

On the question of disposal of radioactive wastes, the Report emphasises that, despite the claims of the nuclear industry, "the whole problem is one of first-rate international importance" and urges that "some internationally acceptable system (be) established for the disposal of high-level wastes, and international supervision of what is done".

Expert witnesses?

The Commissioners made a damaging but probably long overdue attack on the credibility of so-called "expert witnesses", many of whom, it is clear, they heard only with patient sufferance: "In considering the evidence", they say,

we have found that many wildly exaggerated statements are made about the risks and dangers of nuclear energy production by those opposed to it. What has surprised us more is a lack of objectivity in not a few of those in favour of it, including distinguished scientists.

From the published list of witnesses, it is not hard to identify the latter group

In noting the strong emotional overtones of the evidence, the Commissioners found that "distinguished nuclear scientists" were to be found flatly opposed to each other, but "the final decisions should rest with the ordinary man and not be regarded as the preserve of any group of scientists or experts, however distinguished". And in a further attempt to prick the hardened skins of the nuclear lobby, the Commissioners say that "a few of the publicists for nuclear development characterise their opponents as lobbyists or dissidents, or worse". They go on:

We would wish to make it quite plain that before us the opposition has come from a wide cross-section of the general community, and we would not be prepared to conclude that their motives and methods are any less worthy or proper, or intelli-

Fox findings in full

The Fox Report's findings, reflecting the summarised evidence and its analysis of that evidence, may remain its most lasting contribution to an ever-widening debate. In full, they read as follows:

These findings and recommendations are to be read and understood in the context of the Report as a whole and with particular reference to the sections of the Report in which they are respectively discussed

1. The hazards of mining and milling uranium, if those activities are properly regulated and controlled, are not such as to justify a decision not to develop Australian uranium mines.
2. The hazards involved in the ordinary operations of nuclear power reactors, if those operations are properly regulated and controlled, are not such as to justify a decision not to mine and sell Australian uranium.
3. The nuclear power industry is unintentionally contributing to an increased risk of nuclear war. This is the most serious hazard associated with the industry. Complete evaluation of the extent of the risk and assessment of what course should be followed to reduce it involve matters of national security and international relations which are beyond the ambit of the Inquiry. We suggest that the questions involved are of such importance that they be resolved by Parliament. In Chapters 15 and 16 we have gone as far as the terms of reference and the evidence permit in examining the courses open and in making suggestions.
4. Any development of Australian uranium mines should be strictly regulated and controlled, for the purposes mentioned in Chapter 16
5. Any decision about mining for uranium in the Northern Territory should be postponed until the Second Report of this Commission is presented
6. A decision to mine and sell uranium should not be made unless the Commonwealth Government ensures that the Commonwealth can at any time, on the basis of considerations of the nature discussed in this Report, immediately terminate those activities, permanently, indefinitely or for a specified period.
7. Policy respecting Australian uranium exports, for the time being at least, should be based on a full recognition of the hazards, dangers and problems of and associated with the production of nuclear energy, and should therefore seek to

limit or restrict expansion of that production.

8. No sales of Australian uranium should take place to any country not party to the Non-Proliferation Treaty. Export should be subject to the fullest and most effective safeguards agreements, and be supported by fully adequate back-up agreements applying to the entire civil nuclear industry in the country supplied. Australia should work towards the adoption of this policy by other suppliers.

9. A permanent Uranium Advisory Council, to include adequate representation of the people, should be established immediately to advise the Government, but with a duty also to report at least annually to the Parliament, with regard to the export and use of Australian uranium, having in mind in particular the hazards, dangers and problems of and associated with the production of nuclear energy.

10. The Government should immediately explore what steps it can take to assist in reducing the hazards, dangers and problems of and associated with the production of nuclear energy.

11. Policy with regard to the export of uranium should be the subject of regular review.

12. A national energy policy should be developed and reviewed regularly.

13. Steps should be taken immediately to institute full and energetic programs of research and development into (a) liquid fuels to replace petroleum and (b) energy sources other than fossil fuels and nuclear fission.

14. A program of energy conservation should be instituted nationally.

15. The policy of the Government should take into account the importance to Australia, and the countries of the world, of the position of developing countries concerning energy needs and resources

Our final recommendation takes account of what we understand to be the policy of the Act under which the Inquiry was instituted. It is simply that there should be ample time for public consideration of this Report, and for debate upon it. We therefore recommend that no decision be taken in relation to the foregoing matters until a reasonable time has elapsed and there has been an opportunity for the usual democratic processes to function, including in this respect, parliamentary debate.

gently conceived, than, in general, are those of the supporters of nuclear development

Political postscript

The 300 or so people at the Mary Kathleen mine in Queensland, the only active uranium producer at the moment, are dependent on the mine's continuance for employment and are now assured of a job for a few years while the existing contracts are fulfilled. But the investment-led recovery of the national economy, in which the hoped-for expansion of the mining industry played such a key role, has been thrown into confusion since the Fox Report and the decision to export. The massive 17½% devaluation at the

end of November, followed in only 9 days by a 2% revaluation and mostly inconsequential tariff cuts, have rocked the government and its traditional supporters in industry and the press

The style of Mr Fraser was to purvey a well-controlled image of businessman-like decisiveness, repairing the damage allegedly caused by Mr Whitlam's Labor administration. Suddenly, and by its own hand, the government seems vulnerable. If a uranium debate really gets off the ground in 1977 following the Fox Report No. 2, it is anyone's guess as to whether the government will have things all its own way as it did with Report No. 1. □

news and views

Arrangement of immunoglobulin genes

from Alan R. Williamson

THE complete sequences of the first two light chains of human immunoglobulin determined by Hilschmann and Craig (*Proc. natn. Acad. Sci., U.S.A.*, **53**, 1403–1409; 1965) showed that the unique features of each chain lay in the N-terminal half whereas the C-terminal halves of the two chains were identical. Extensive sequence data from both light and heavy chains have since shown that each chain has a unique N-terminal sequence (variable region) of about 110–120 amino acids. The hypothesis that two genes code for each immunoglobulin chain was advanced by Dreyer and Bennett in 1965 to account for these data, and patterns of inheritance have supported the proposal that a single constant (C) region gene is expressed in conjunction with any one of a set of variable (V) region genes.

The question then arises of the stage at which the information from the V and C genes is combined to produce the complete antibody chain. Evidence against joining at the polypeptide or RNA levels has steadily accumulated. Contiguous translation of V and C regions from the same mRNA (Milstein *et al.*, *Nature*, **252**, 354; 1974) and the absence of V and C scrambling in hybrid cells (Kohler and Milstein, *Nature*, **256**, 495; 1975) both fit more easily the hypothesis that the V and C genes are rearranged and joined together in the genome.

Evidence that the physical arrangement of V and C genes differs in the genome of embryonic and somatic cells has now been presented by Hozumi and Tonegawa (*Proc. natn. Acad. Sci. U.S.A.*, **73**, 3628; 1976). The experiment is elegantly simple in concept; there are however limitations to the interpretation. DNA (either from BALB/c mouse embryos (12 or 13 d old) or from the BALB/c myeloma tumour MOPC321) is specifically fragmented with the restriction endonuclease *Bam*H1. The fragments are separated electrophoretically on slabs of agarose which are then cut into sections and the DNA extracted from each one. Each fraction of DNA is tested for sequence complementarity to κ -chain mRNA prepared from MOPC321 tumour cells.

Different patterns of complementary DNA fractions were found with embryo and tumour DNA. In a digest of embryonic DNA, two fragments (about 6.6 and 9.2 kb) showed significant hybridisation with the κ 321 mRNA probe whereas a digest of tumour DNA showed significant hybridisation to only one fragment (about 3.5 kb). The peaks of hybridisation are broad and only a fraction of input radioactivity was hybridised (<20%). Identification of the peaks is made using a second probe specific mainly for the C region of κ chain. This probe was prepared after the fragmentation of mRNA during iodination by selection of the appropriately sized 3'-end fragments containing poly(A). By this test the 9.2 kb fragment of embryonic DNA contains C_K sequences but no V_{321} sequence whereas the 6.6 kb fragment contains V_{321} sequence but no C_K sequence. The 3.5 kb fragment of tumour DNA apparently contains both C_K and V_{321} sequences. These results are interpreted by the authors as showing that V_{321} and C_K genes are located separately in embryonic DNA but occur in the same short stretch of 321 tumour DNA. Clearly this interpretation is consistent with somatic rearrangement of V_{321} and C_K to give a contiguous gene. Alternative explanations involving two short stretches of DNA in 321 (one V_{321} and one C_K) or mutations introducing and removing *Bam*H1 sites are considered less probable than the contiguous gene hypothesis.

It is tempting to accept that somatic rearrangement of V and C genes is now beyond reasonable doubt and to seek for some mechanism. But it is when one comes to consider the possible mechanisms that the present data appear too simple in the light of some of the other evidence available from studies of immunoglobulin genes in myeloma cells.

Immunoglobulin-bearing cells are functionally haploid (that is, only one allele is expressed). The restriction mapping, which results in only one type of fragment homologous to the V or C probes in the tumour cell, suggests therefore that either MOPC321 cells are physically haploid or that some form of homozygosity has occurred yielding homologous

chromosomes with the same VC pair on both. There is also the complication that myeloma cells are usually aneuploid but the authors do not comment on this point. The C_K gene seems to be in a linkage group on chromosome 6 in the mouse, so knowledge of the number of copies of this chromosome in 321 tumour cells would clarify the interpretation.

Studies of somatic mutation in myeloma proteins suggest that myeloma cells have only one expressed VC_K gene (Milstein *et al.*, in *Molecular Approaches to Immunology*, edit. by Smith, E. E., and Ribbons, D. S., 131–148, Academic, New York and London, 1975) although some myeloma tumours possibly express an additional C_K gene (Kuehl *et al.*, *Cell*, **5**, 139; 1975) and there is also evidence for more than one C_K gene per haploid genome (Stavnezer *et al.*, **88**, 43; 1974). The absence of C_K restriction fragments in myeloma DNA is therefore puzzling and a single $V_{321}C_K$ restriction fragment may be too simple an interpretation of the data. Alternatively MOPC321 may be an unusual tumour which has lost all but the functional C_K gene.

A different way of asking the rearrangement question would be to map the DNA of a tumour other than 321 (that is, not expressing V_{321}) using the V_{321} mRNA probe. Analysis of 321 tumour DNA with another κ mRNA would also be valuable. In these experiments the relative locations of expressed and non-expressed V region sequences could be determined on the same DNA digest.

Hozumi and Tonegawa comment that their choice of hybridisation technique was limited by the purity of κ mRNA currently achievable. Mach and Rougeon reported at the 9th Harden Conference the successful cloning of κ cDNA in a bacterial plasmid (see *News and Views*, **263**, 726; 1976). The availability of homogeneous nucleic acid probes will make possible the application of hybridisation techniques using excess probe; even 90% purity of probe is insufficient for such techniques.

Conclusive evidence for somatic rearrangement of antibody genes may not be far away. Knowledge of the mechanism is probably a little more remote. □

Regulating steroid action of the molecular level

from Robert Shields

EVERY budding endocrinologist learns the current dogma of steroid action at his Alma Mater's knee. The patter runs something like this. After entering the cell steroids bind to specific receptors (present only in responsive cells) which migrate to the nucleus, switch on specific genes and so increase cytoplasmic mRNAs coding for specific proteins. While there is good evidence for the first and last parts of the scheme, what happens in the nucleus is still confused.

It is clear in some cases at least that the steroid hormones can be very selective in how many of the cell's 1×10^4 – 3×10^4 different mRNA species they control. For instance, in the *Drosophila* polytene chromosomes the pattern of gene puffing induced by ecdysone shows that this steroid hormone controls relatively few genes (Ashburner *et al.*, *Cold Spring Harbor Symp. quant. Biol.*, **38**, 655, 1973). In other systems direct measurements of mRNA species suggest that only a limited number of sequences are steroid regulated. Experiments using DNA complementary to purified ovalbumin mRNA have shown that oestrogen induction of ovalbumin in the chick oviduct involves an enormous increase in ovalbumin mRNA (McKnight *et al.*, *J. biol. Chem.*, **250**, 8105; 1975; Harris *et al.*, *Biochemistry*, **14**, 2072; 1975) and less direct experiments suggest that the mRNA for conalbumin also increases (Palmiter *et al.*, *Cell*, **8**, 557; 1976). But these experiments do not indicate how many other proteins are affected by oestrogens.

An approach to this problem is to analyse the mRNA complexity in tissues of different hormonal status by measuring the kinetics of hybridisation of total mRNA to its complementary DNA. Hybridisation of excess mRNA isolated from hormone-withdrawn or oestrogen-stimulated chick oviduct to its respective complementary DNA showed that oestrogen treatment led to the synthesis of many thousands of copies of between one and ten mRNA species (Axel *et al.*, *Cell*, **7**, 247; 1976; Monahan *et al.*, *J. biol. Chem.*, **251**, 3738; 1976). This handful of species includes ovalbumin mRNA (Axel *et al. op. cit.*) and presumably the mRNA species for other egg white proteins as well.

Within the margin of error of this type of homologous hybridisation it is impossible to state definitely whether oestrogens induce significant numbers of other mRNA species that are

normally present in only a few copies per cell (low abundance). Heterologous hybridisations using mRNA isolated from oviduct in one hormonal state and cDNA prepared from mRNAs from another hormonal state should be able to decide this question. Where such heterologous hybridisations have been done (rat prostate) the major effect of the steroid hormone (testosterone) seems to be the increase in the number of copies of relatively few mRNA species (M. Parker, personal communication). Further support for the very limited action of steroids comes from experiments where the total proteins synthesised in glucocorticoid-treated hepatoma (HTC cells) were examined by two-dimensional electrophoresis. Here again it is apparent that only a handful of the cell's proteins including that old stalwart tyrosine transaminase are affected by the steroid (R. Ivarie and P. O'Farrel, personal communication). In all this it should not be overlooked that while steroids may alter the relative synthesis of only a few mRNAs they may also exert general effects on the total amount of RNA present in the cell.

How does the receptor-steroid complex bring about these relatively specific changes in a few mRNAs? By analogy with prokaryotes, in which gene control is understood rather better, receptors could bind to specific regions on the eukaryotic chromatin similar to a bacterial operator leading to increased gene transcription. The analogy would demand that the nuclear gene control sites would be limited in number (comparable to the number of genes controlled by the steroid) and the binding of receptor to these sites would be specific (that is, it would have a higher affinity for control than non-control regions of the genome). Furthermore, any explanation of steroid regulation should account for the fact that there are around 10^4 receptors per cell (although only 10 or fewer genes are steroid regulated).

Measurements of the numbers of nuclear binding sites for receptors have provided conflicting results. *In vivo* there is a good correlation between biological effect, steroid receptor binding and transfer of the receptor steroid complex to the nucleus (Katzenellenbogen and Gorski, *J. biol. Chem.*, **247**, 1299; 1972). The proportion of cellular steroid complexes in the nucleus is, however, independent of the dose of administered steroid (Williams and Gorski, *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3464; 1972), a result that is incon-

sistent with the presence of a strictly limited number of nuclear binding sites for the receptor. Instead the results point to at least a 5–10-fold excess of nuclear binding sites over steroid receptors, approaching 10^5 sites per cell, far exceeding the number of genes under steroid control. As yet there are only a few eukaryotic mutants that are of much use in dissecting the steroid-mediated events in the cell nucleus so attention *in vitro* of steroid receptors and isolated nuclei of nuclear subfractions. While a great many results show that the number of nuclear binding sites for receptor may be very large (Chamness *et al.*, *Biochemistry*, **13**, 327; 1974; Simons *et al.*, *J. biol. Chem.*, **251**, 339; 1976) several other *in vitro* experiments suggest that this is not the case (Higgins *et al.*, *J. biol. Chem.*, **248**, 5866; 1973; Rousseau *et al.*, *J. Steroid Biochem.*, **5**, 935; 1974). Even if the lowest number for nuclear binding sites is accepted ($\sim 15,000$) this is at least two orders of magnitude greater than the number of controlled genes.

One way out of this dilemma has been suggested recently (Yamamoto and Alberts, *Cell*, **4**, 310; 1975). Their proposal is that steroid-receptor complexes bind with low affinity to a very large number of nonspecific sites in the cell DNA. This nonspecific binding is the binding that is measured in *in vitro* experiments and completely masks the binding of receptor to up to 10^5 specific sites of high affinity which are the true control regions. The model is strongly influenced by studies of the *lac* operon in *E. coli*. Here it was known on genetic grounds that the repressor bound to gene control sites but specific repressor binding could not be demonstrated to *E. coli* DNA. Only when the *lac* operon was purified by incorporation in phage could specific repressor binding be demonstrated (Lin and Riggs, *J. molec. Biol.*, **72**, 671; 1972). This theory also neatly accounts for the apparently large number of receptors in the cells. They are present in large quantities so that enough receptors are left to bind to control loci when the rest are mopped up by binding to nonspecific regions. Perhaps the, nonspecific binding to multiple sites is the biological price that has to be paid to get reversible receptor binding to a few specific control elements. But, if the measured receptor binding sites (the nonspecific sites) consist only of DNA as has been suggested (Yamamoto and Alberts, *op. cit.*) there should be no specificity of receptor binding to tar-

get and non-target nuclei. Reports on this point are conflicting, both tissue specificity (Higgins *et al.*, *J. biol. Chem.*, **248**, 5873, 1973) and lack of specificity (Chamness, *op cit.*) have been reported

Perhaps the situation will only be finally resolved when purified receptor is available and it is possible to clone the DNA fragments containing the controlled gene and its operator region and so study receptor binding *in vitro*. Two systems are presently being explored in this manner, the control of ovalbumin synthesis in the oviduct (Woo *et al.*, *J. biol. Chem.*, **251**, 3868; 1976) and the glucocorticoid regulation of mouse mammary tumour virus (see *News and Views*, **259**, 193; 1976). □

Fish schooling

from John Krebs

Two recent papers have shed interesting new light on fish schools. Many species of fish swim in polarised and coordinated groups containing anything from half a dozen to many thousands of individuals, according to the species. Pitcher *et al.* (*Science*, **194**, 963; 1976) have investigated the sensory mechanisms involved in schooling, and Breder (*Fish Bull.*, **74**, 471; 1976) has suggested a new functional explanation for the schooling habit.

In the past, several workers have emphasised the importance of vision in schooling. schools of various species tend to break up at low light intensities. Pitcher *et al.* studied the schooling abilities of Saithe (*Pollachius virens*) fitted with black plastic eye-cups. These temporarily blind fish could be scooped out of the water showing no attempt to avoid the net, but could still join and maintain their position in a fish school swimming around a large circular tank. The blind fish showed some small differences in behaviour: they moved from side to side within the school more than their companions, but in the Saithe at least, vision is clearly not necessary for schooling. Pitcher *et al.* go on to show that if the lateral line system of pressure detectors is cut, the blind fish no longer school, which seems to show that the blind Saithe use water movements created by their companions in order to stay in position in the school.

The adaptive significance of schooling is also much debated and several hypotheses (not mutually exclusive) have emerged. One is that schools are an anti-predator adaptation, the idea being that the individuals in a school "seek cover" from potential attackers by hiding behind a fellow schooler

(Hamilton, *J. theor. Biol.*, **31**, 295; 1971). This might be called the "after you" principle. Experiments have shown that predators also tend to be confused when faced with a milling school of prey, so that attack success goes down (Neill and Cullen, *J. Zool. Lond.*, **172**, 549; 1974). Another possible benefit of being in a school is that each individual is able to parasitise (by copying) the success of its companions in finding good feeding places an effect well known to fishermen and blackberry pickers. A less well-documented possibility is that there may be a hydrodynamic, energy saving, advantage of schools. As a result of its swimming movements, each fish produces a series of vortices in the water to either side of its body, and if a following fish swims in exactly the right spot, it could in theory get a forward boost from the vortex, allowing it to free wheel and save energy (Weihs, *Nature*, **241**, 290; 1973). Only the hapless individuals at the front gain nothing, but fish are known to jostle for positions at the back and centre of a school, and this hydrodynamic theory may explain why. Breder also suggests a hydrodynamic advantage which would benefit all but the front swimmers. He notes that the mucus produced by the bodies of fish has an important effect in reducing frictional drag. In some species even a 5% solution of mucus can reduce drag by up to 60%. Breder's idea is that fish in a school (except for the front swimmer) benefit not only from their own mucus, but from the slime washing off the bodies in front of them. He suggests that schooling may have originated simply as a consequence of fish finding it energetically easier to swim along behind someone else.

Breder's suggestion is an addition rather than an alternative to the anti-predator and feeding advantages of schooling. In fact, to explain the differences between species of fish in their tendency to form schools, one will have to look for ecological factors such as the risk of predator attacks, since the hydrodynamic advantage probably would apply roughly equally to all fish. □

Troubled waters

from Peter D. Moore

THE art of compromise may not be dead, but it is certainly not thriving, judging by the views put forward at a conference (Recreation and Conservation in Water Areas) held by the Royal Society of Arts in London on November 22. The meeting succeeded in demonstrating that there is little common ground, or water, between the

two interests. Such a state of affairs is predictable, for bird watchers and their ilk have never been noted for their affection towards water-skiers and power-boat drivers, but what does seem remarkable is the total lack of understanding which each group of water-users has of the others interests and requirements. The problem is a very real one for the regional Water Authorities, which were set up under the 1973 Water Act. This same Act makes these Authorities responsible for making their waters and associated lands available for public recreation (Section 20) and also places statutory obligation upon them to grant public access and to have regard to conservation interests (Section 22). Whether they like it or not, the Water Authorities must try to satisfy all demands.

B. Hackett (University of Newcastle-upon-Tyne) represented the landscape designer's point of view and evidently found considerable aesthetic appeal in the geometrical, concrete-edged lake within a built landscape setting. Many may share his view, but he cannot expect enthusiasm from the angler or the wildlife conservationist; neither can he expect these groups to be responsive to his 'semi-natural' compromise, which is a body of water surrounded by carefully selected shrubs and neatly planted flower beds. For the planner, I suppose, nature can only be attractive when planned.

Then there is the true recreationist. Does N. R. Collins (Sports Council) really believe that the disturbance created by water skiers reoxygenates water, or was that a joke? Even if it were demonstrably true I cannot imagine the three million odd British anglers being very impressed. There is no chance of compromise here.

C. Newbold (Nature Conservancy Council) presented a distinctly uncompromising statement of the conservationist's position. Quoting the work of Hume (*Br Birds*, **69**, 178; 1976) he explained that a power boat puts diving ducks (the goldeneye for example) to flight at a range of 500-700 m, sailing boats do so at 350-400 m and people on the bank at 100-200 m. He rather assumed that the latter would be anglers, but they could equally well be bird watchers themselves. The figures do, however, serve to highlight the conflict of interests between bird watchers and just about all other water users. The vociferous and adverse audience reaction to Newbold's suggestion that the construction of a restaurant on the shores of the Loch Insh Nature Reserve was a retrograde step in terms of amenity, ably demonstrates that 'amenity' can mean different things to different people.

Wildfowling have had much experience in respecting the needs of others

and J. Harrison (Wildfowlers' Association of Great Britain and Ireland) presented a broad and sympathetic survey of the problems of water use. Anglers and bird watchers can be reconciled, he claimed, if the anglers are restricted to specifically designed sites on the bank, screened by trees and bushes. I wonder how the anglers react to such a screen when trying to make a cast.

Reconciliation is still a long way off. Zoning of the larger lakes, such as Loch Lomond and Llyn Tegid in which certain activities are restricted to specific areas, is successful at some sites, but requires unselfish cooperation from all water users. Smaller sites may have to be reserved for single activities. This will still create arguments over priorities, for England and Wales contain only 300,000 acres of water space and conservationists assure us that 94% of southern grade 1 and grade 2 conservation sites are currently used for recreation. □

Tsetse ecology and behaviour

from a Correspondent

A Study Workshop on the ecology and behaviour of tsetse flies was held in Nairobi on September 28—October 2, 1976. It was organised by Professor T. R. Odhiambo, Director of the International Centre of Insect Physiology and Ecology, Nairobi.

THE objectives of the workshop were to assess the state of current knowledge in this field, and on that basis to formulate plans for future research with specific reference to the possible development of acceptable methods of controlling the tsetse vector of trypanosomiasis.

Scientific sessions opened with a review of current knowledge of tsetse ecology by A. M. Jordan (University of Bristol), who emphasised the need for a quantitative appraisal of mortality factors operative in natural tsetse populations. This was followed by accounts of recent work on the ecology of an atypical, peridomestic populations of *Glossina palpalis* in lower Zaire (P. van Wettere, WHO, Upper Volta) and of *G. tachinoides* in West Africa (D. A. T. Baldry, WHO, Geneva); and of typical populations of *G. swynnertoni* in Tanzania (S. K. Moloo, ILRAD, Nairobi), of *G. morsitans morsitans* in Zambia (S. N. Okiwelu and L. C. Madubunyi (University

of Nigeria, Nsukka)) and in Botswana (R. Allsopp, COPR, London), and of *G. pallidipes* in Kenya (J. van Etten, ICIPE). A dominant theme throughout was the possible occurrence of genetically distinct populations within a species, characterised by differences in host preference and other aspects of behaviour which might affect vectorial capacity. With regard to the peridomestic populations which could assume increasing importance in the context of developing Africa, the possibility of reducing challenge by appropriate modifications of livestock management practice, or by limited but highly selective application of insecticides, should clearly be investigated.

Recent developments in tsetse genetics were reviewed by W. Helle (Universitat van Amsterdam), who concluded that empirical studies of tsetse genetics would have an important part to play in the assessment of possible genetic differences between populations, and in guarding against the occurrence of undesirable genetic drift in colonies destined for use in sterile-male release programmes (such as the current Tanga project, which was later described by the project leader, L. Williamson (Tsetse Research Project, Tanga, Tanzania)). L. P. S. van der Geest (Universitat van Amsterdam) presented preliminary results of his isoenzyme investigations, reporting that different laboratory colonies could be shown to differ substantially in the relative frequency of alleles at the leucine-alanine peptidase locus. The importance of extending studies of this kind to natural populations of tsetse flies was agreed.

The session on tsetse behaviour was opened by J. Brady (Imperial College, London), who reviewed work on endogenous rhythms in tsetse flies, and described an analysis of available field data on diurnal patterns of activity in terms of an interplay between endogenous and environmental factors, emphasising the importance of a close integration of field and laboratory investigations. The need for a close study of the "medium range" reactions of tsetse flies to their hosts (as opposed to the long-range olfactory and the short-range thermal and gustatory reactions) was brought out; this, too, was the main theme of G. A. Vale (Department of Veterinary Services, Salisbury, Rhodesia) in his discussion of sampling techniques, where the effectiveness of different sampling methods for different components of the tsetse population and for different species was shown to be closely related to this aspect of behaviour. Vale also reported that investigations were in progress to explore the possibility of using the long-range attraction of tsetse flies to host odours as a basis for a sterile-male

release programme based on the capture, rather than on the rearing, of male flies.

A new dimension was introduced by J. Keiding (Danish Pest Infestation Laboratory, Lyngby), who described aspects of the biology of *Musca domestica* in Denmark, and showed that many features of its biology had been capable of convincing interpretation on the basis of results obtained from a range of relatively simple laboratory experiments. It was agreed that similar work could usefully be carried out with the newly emerged tsetse fly, which might represent a vulnerable stage in the life history. In the subsequent discussion, it was emphasised that in studies of tsetse behaviour due attention should be paid to the possible importance of group or social interactions.

A. Youdeowei (University of Ibadan) presented a paper on the salivation behaviour of the tsetse, based on a newly developed experimental technique, and the possibility was mentioned of interfering with trypanosome transmission at this stage of the cycle. This was followed by a discussion by T. Jaenson (University of Nairobi) of his recent work on the mating of *G. pallidipes*, which has long been recognised as a difficult species from the point of view of laboratory maintenance because of its recalcitrance to laboratory mating. The increased receptivity which characterised the first week in the life of adult females was shown to be associated with a corre-



A hundred years ago

A MEETING was recently held in Birmingham of the Council in connection with the projected aquarium for that town. We are glad to see that the arrangements for carrying the scheme into execution are well forwarded, and a Committee was appointed at the meeting to make all necessary preliminary arrangements. The proposed plan of the aquarium seems to us all that could be desired, and we are glad to see that Mr. Hughes, and other speakers at the above meeting, showed a laudable desire to make the institution serve important educational purposes; we hope, at least, that it will not degenerate into a second-rate music-hall and miscellaneous rendezvous.

From *Nature*, 15, December 21, 170; 1876.

sponding decrease in copulation time; additionally, evidence in support of the existence of genotypically distinct populations of the species was adduced on the basis of differences in copulation time between colonies deriving from different localities. E. Bursell (University of Rhodesia, Salisbury), described recent work on the metabolic basis of flight activity in the tsetse, and presented a source/sink analysis of energy reserves on the basis of which it was concluded that male tsetse flies might spend far more time in flight than had hitherto been supposed, whereas values for the female raised the possibility that slight reductions in host availability might cause a substantial decrease in reproductive potential.

In reviewing the proceedings as a whole, participants agreed that the potential of host odours and pheromones for use in control programmes and in the development of sampling techniques for low density populations deserved careful investigation. They noted, further, that a constantly recurring theme throughout the deliberations had been the need to mount a large-scale, long-term, multidisciplinary investigation of the crucial relation between the tsetse and its hosts in the natural environment, with particular reference to the quantitative assessment of mortality factors operating on a natural tsetse population. Only in this way could a basis be provided for the better understanding, in quantitative terms, of factors governing the population dynamics of the species, without which the potential for the development of integrated control programmes would be incapable of proper assessment. In this connection it was emphasised that the activity of tsetse control should not be allowed to take on "a life of its own", but that it should be seen as just one element in the broader context of the proper management of natural resources. □

Ecdysone binding across the Rhine

from David Whitehead

An informal Ecdysone Workshop was held at the Royal Entomological Society, London on November 11, 1976.

ECDYSTERONE, it is understood, elicits its manifold response by entering the cells of the target tissue whereupon it is carried to the nucleus by specific cytosol proteins where it may or may not interact with a DNA repressor on

the genome. Whereas the evidence for this type of model may now be good for mammalian steroid hormones, invertebrate biochemists are just beginning to make headway again.

At the meeting laboratories in Germany and France revealed their up-to-the-minute findings concerning the specific binding of the 'ecdysteroids' ecdysone and ecdysterone (the arthropod moult hormones) to various tissues of a locust (J. A. Hoffman, Université Louis Pasteur, Strasbourg), a crayfish (K.-D. Spindler, Technische Hochschule Darmstadt) and to fruit fly salivary glands (H. Emmerich, Darmstadt).

Hoffmann and colleagues have found 'receptors' for ecdysterone in epidermis, fat body and even muscle. Maximal binding was achieved at 10^{-7} M, which happens to be the maximum titre in *Locusta migratoria* as detected by bioassay and by the radioimmunoassay developed in Marseilles (de Reggi *et al.*, *Biochem. biophys. Res. Commun.*, **66**, 1807; 1975). Specific binding of ecdysone occurred in the epidermis only. This supports the contention (P. Karlson and J. Koolman, Philipps Universität, Marburg) that ecdysone is not just a prohormone but is an active primary messenger in its own right. Difficulties have arisen, however, in quantifying binding to the various 'receptors' because the parent tissues were derived from locusts with high endogenous titres of ecdysteroids. Unfortunately, prothoracic gland extirpation or selective X irradiation of larvae block the activation of the binding sites themselves. Therefore, the task of characterising the 'receptors' is necessarily a slow one; for instance, the exact amount of hormone in the nuclei and in the cytosol must be known for each tissue used.

Saturable 'cytoplasmic receptors' for ecdysterone ($\sim 140,000$ daltons) were demonstrated in hypodermis, hindgut and gonads of *Orconectes limosus* using equilibrium dialysis, charcoal assays and equilibrium density gradient centrifugation (Kuppert and Spindler). After partial purification, binding molecules with high affinity for ecdysterone were found in the cytoplasm of salivary glands from mid stage III larvae of *Drosophila* (Beckers and Emmerich). Outside the target cells R. Lafont and coworkers (Ecole Normale Supérieure, Paris) suggested that ecdysteroids could be bound reversibly to small polar components in haemolymph which bore a striking resemblance to the fat body factor of G. Bergstrom and H. Oberlander (*J. Insect Physiol.*, **21**, 39; 1975).

H. Rees (University of Liverpool) reported that ecdysone C-20 hydroxylase from *Schistocerca* Malpighian tubule mitochondria is a mixed func-

tion oxidase involving P-450 cytochrome. Peak activity of the enzyme is reached just before the maximum hormone titre in the 5th instar larva implying that the hydroxylase might regulate the ecdysterone level. Koolman was concerned that metabolism of both ecdysone and ecdysterone into the 3-dehydro derivatives (followed by epimerisation) and into the polar conjugates (glucuronides, glucosides, sulphates) could effectively regulate the titre of moulting hormone in blowflies and locusts. There were, however, demonstrable differences in the activity of the various enzymes between species and even between stages of the same insect. For ecdysone oxidase, a cytosol enzyme in *Calliphora* larvae, Koolman showed that three factors regulate the level of hormone: the enzyme titre, its availability (compartmentation in the cell) and its kinetic properties. (Since the meeting new evidence has come to light that 3-dehydroecdysone—the product of ecdysone oxidation—may be active itself in some systems. It initiated puffing in *Drosophila* salivary glands (G. Richards, University of Cambridge) and yet it did not cross react with rabbit antisera to ecdysone carboxymethylloxime-BSA complex (D. Whitehead, University of Bristol)).

Richards presented evidence of ecdysone-induced chromosomal puffing in the salivary glands of *D. melanogaster* using *in vitro* analysis. He suggested that ecdysteroid titres may fluctuate rapidly in normal development. In particular, in the 12–14 h of the prepupal stage, effective titres of ecdysterone may change from 10^{-7} M to 10^{-9} M and back to 10^{-7} M in the salivary gland. Although radioimmunoassay has recently allowed an investigation of hormone titres previously not possible in fruit flies, total values found may be misleading since biologically inactive ecdysteroids could cross react in the system. For instance, the high titres found *in vivo* 3–8 h after puparium formation would inhibit the normal development *in vitro* of the salivary gland at this mid-prepupal stage. His suggestion was that either the hormone is sequestered *in vivo* so as to reduce the effective titre or it is metabolised to biologically inactive products.

Puparium formation of *D. lebanonensis* is under the control of a circadian oscillation. J. Eeken (Nijmegen, Holland) showed that this oscillation allows pupariation to occur only during specific 'gate' periods every 24 h (L:D=12:12). Injection of a group of larvae with ecdysterone before the 'gate' period which preceded pupariation by 24 h, induces specific puffing in the polytene chromosomes of salivary glands. Injection after the 'gate' is

also followed by puffing but not by pupariation. The result is not due to supplementation of endogenous titres by injection because no ecdysteroids are detectable using the new antisera (Horn *et al.*, *J. Insect Physiol.*, **22**, 901; 1976) before or immediately after the 'gate' period. Hormone titre only increases when pupariation commences where the level is $0.11\text{--}0.15\text{ nmol g}^{-1}$. Similarly Whitehead found that stage III tsetse fly larvae are devoid of hormone during sclerotisation of the poly-pneustic lobes *in utero* as well as during and after birth. The radioimmuno-assay first detected ecdysteroids during pupariation and the titre rose eventually to $0.8\text{--}0.9\text{ nmol g}^{-1}$ after pupal-adult apolysis.

A more practical observation was that 10^{-8} M ecdysterone and inokosterone added to blood fed through a membrane to *Glossina morsitans* females after ovulation caused 55–80% of the eggs and larvae to be aborted. The systemic action may be at the level of the Malpighian tubules (which were activated *in vitro* to secrete urine (Gee, Koolman and Whitehead)) and the uterine glands which nourish the larva, rather than directly on the offspring.

P. Berreur and coworkers (CNRS, Gif sur Yvette, France) demonstrated a simple device for positioning blowfly larvae before ablation of the ring gland complex containing the gland responsible for ecdysone synthesis. Twelve days after the operation the permanent larvae which result contained 0.08 nmol g^{-1} of ecdysteroids whereas the titre at pupariation was normally ten times this level. Furthermore, wing disks of the ablated larvae were unable to incorporate ^3H -uridine normally after injection into the larva. □

Parasite invasion

from V. R. Southgate

The fifteenth meeting of the British Society of Parasitology, entitled Parasite Invasion was held at the Zoological Society of London on October 29, 1976.

PARASITES must, at some stage in their life-cycle, move from one host to another, then find appropriate sites within the host to which they become morphologically, physiologically and biochemically adapted. To do this often requires penetration. Penetration mechanisms of parasites are relatively poorly understood, although recent advances have revealed glimpses of the levels of complexity involved. Therefore it seemed appropriate to review

current knowledge and ideas on the mechanisms of parasite invasion and the accompanying physiological changes which take place. It is of fundamental importance to understand these diverse processes which parasites, in order to be successful, have evolved in association with their hosts.

Parasites attach to host cells/tissues in many ways, and the degree of specificity controlling attachments and invasion varies. For example, P. L. Long (Houghton Poultry Research Station) and C. A. Speer (University of Montana) reported that sporozoites of coccidia will enter a wide variety of cell types *in vitro*. However, *in vivo* studies demonstrate that site specificity is strong although factors influencing this are unknown. In contrast, L. H. Bannister (Department of Biology and Anatomy, Guy's Hospital) reported that the merozoite of *Plasmodium* will adhere to several cell types, but penetration only occurs when the apical surface of the merozoite contacts a red blood cell membrane. Thus, invasion depends upon the recognition of specific receptors, and interest has been aroused in the possible relationships of the receptors of *P. vivax* and *P. knowlesi* with blood group antigens of the Duffy class (Miller and Carter, *Expl Parasitol.*, **40**, 132; 1976). The signal for invasion remains elusive. Different groups of viruses have assorted methods for cell attachment and entry: for example, D. A. J. Tyrrell (Clinical Research Centre, Harrow) indicated that influenza viruses attach to cells by 'interlocking' viral glycopeptides to host cell neuraminic acid residues. If attached to cells which are unsuitable for penetration, these viruses are able to detach themselves by secreting neuraminidase. Helminths perceive certain stimuli through complex sensory receptors which apparently initiate behavioural patterns such as release of cytolytic secretion. The schistosome cercariae which invade man respond to rise in temperature and fatty acids of the host skin (Schiff *et al.*, *J. Parasitol.*, **58**, 476; 1976).

Thus, evidence is increasing to suggest a molecular basis for attachment and signals for invasion, which in turn is helping to explain specificity.

What are the actual mechanics of invasion? Our knowledge of the processes involved are limited, especially in the protozoa. It seems likely that well-defined cell organelles are important. Cinemicrographic studies suggest invasion is an active process on the part of the sporozoite of coccidia, and the conoid (a truncated hollow cone of 6–8 spirally arranged fibrils) is capable of protrusion and contraction. The merozoite of *Plasmodium* somehow induces the host cell membrane to in-

vaginate: the invagination 'sucks' the merozoite inwards. Possibly, substances secreted from the rhoptries and micronemes (merozoite organelles) are absorbed into the host cell membrane, thereby altering its biophysical properties. B. E. Matthews (University of Wales, Bangor) demonstrated that helminths pass through tissue barriers using one or a combination of three methods: mechanical invasion, the use of cytolytic secretions; or normal processes, such as feeding.

Why are there alterations in metabolic pathways after invasion? J. Barrett (University of Wales, Aberystwyth) considered two factors to be of major importance: the metabolism of infective helminth larvae is geared to survival whereas that of adults is directed towards reproduction. Also, the physical environment is different, for example, in homeotherms, the temperature and partial pressure of CO_2 rises, and the partial pressure of O_2 falls. Basically, the metabolism of infective larvae is aerobic whilst that of adult parasites is anaerobic—the switch from one to another is controlled genetically and by physical factors. P. I. Trigg (National Institute of Medical Research, Mill Hill) and W. Gutteridge (University of Kent) argued that difficulties associated with *in vitro* culture and separation of protozoan parasites from their host cells for biochemical analysis are the two main reasons for the incomplete picture of physiological and biochemical changes during life-cycles. These problems are not so acute with the extracellular trypanosomes. Consequently, the morphological and metabolic differences between the vertebrate blood and insect midgut forms are comparatively well-documented. Interpretation of the physiological and biochemical changes in coccidia and *Plasmodium* have been based largely on ultrastructural and cytochemical observations, but the recent advances in the continuous cultivation of *P. falciparum* by Trager and Jensen (*Science*, **193**, 673; 1976) and Haynes *et al.* (*Nature*, **263**, 767; 1976) may help to rectify this imbalance.

Advances in techniques in the future will enable a more precise identification of specific receptors at the molecular level on both parasite and host to be attempted.

Parasitologists will also be trying to uncover the nature of signals which initiate behavioural patterns. Advances in *in vitro* culture of protozoan parasites and use of natural barriers *in vitro* for infective larvae will make collection of material for biochemical analyses easier, but problems of clean separation of intracellular protozoa from host cells will have to be overcome. □

review article

Selective stabilisation of developing synapses as a mechanism for the specification of neuronal networks

Jean-Pierre Changeux & Antoine Danchin*

The specificity of synapses in the mammalian brain cannot possibly be accounted for by one-to-one biochemical matching. The alternative proposed in this article is that connections are genetically specified between classes of cells, but the final wiring pattern depends on the refinement of those collections by selective stabilisation during neuronal activity.

In his 1951 Ferrier lecture¹, J. Z. Young proposed that the mature synapse represents the end product of a continuous process of growth and degeneration of the terminal part of nerve fibres and further suggested that the "modification of such daily growth by functional activity would provide the basis of the plasticity of the nervous system". Since then, the portrait of the synapse has been drawn in more detail with the addition, in particular, of its fine organisation², the elementary mechanisms of its activity^{3,4}, the chemistry of some fine critical components⁵ and the role of factors which selectively affect the growth of nerve terminals⁶. In parallel, and independently, the basic structural features of the genetic material and of its expression have emerged. Encouraged by the success of genetic analysis in the acquisition of this knowledge, molecular biologists interested in the nervous system have focused primarily on its genetic determination^{7,8} and have thus raised the question of how such determination takes place. The DNA present in the nucleus of the fertilised egg may, at most, code for only a few million proteins and this quantity is practically the same in mouse, chimpanzee and man, moreover a significant fraction of it (more than 60%) does not correspond to genuine structural genes⁹. How is it then possible to engender the formidable complexity of the nervous system from such a limited number of genes?

The answer must lie in the mechanics of embryonic development. Models have been proposed which, from the combination of a small number of genes expressing themselves sequentially, and a few yes-no signals, produce a significant diversity of cell types¹⁰. They offer plausible, but still theoretical, explanations for the setting out, for example, of the neuronal somas. The establishment of adult connectivity, particularly of the vertebrate nervous system, is of a higher order of complexity. The call for "gene saving" mechanisms becomes pressing. The hypotheses which have been put forward to explain how nerve cells become interconnected during development differ chiefly on the question of whether the functional activity of the developing network plays a part. Schematically, the two main conceptual attitudes are the following.

(1) For Sperry¹¹ ("cytodifferentiation" or "chemoaffinity" hypothesis) the nerve fibres in the process of growth bear chemical labels complementary to those of their neuronal target; each label reflects the position of a given cell within a set. The pre- and postsynaptic partners recognise and assemble

themselves with a selectivity which depends on the complementarity between cell surfaces. Gaze and Keating¹² and Prestidge and Willshaw¹³ have amplified and extended this cytodifferentiation hypothesis introducing, in particular, a graded affinity between axons and postsynaptic targets and a competition between ingrowing nerve terminals and their postsynaptic partners, both being present in limited numbers. Gaze¹⁴ and Jacobson¹⁵ have also considered temporal factors in the establishment of connections. The differential growth of nerve fibres would be instrumental in bringing an order: reaching their target neurones at different times, the nerve terminals would "number" them in sequential order ("timing" hypothesis). The plausibility of these hypotheses has already been discussed^{12-15,16}.

(2) Since Ramon y Cajal¹⁷ and others, three attitudes have been adopted; briefly they are the following (Fig. 1).

First, as in the "preformist" views just mentioned, the neuronal network is assumed to be specified before experience. Interaction with the environment merely triggers pre-established programmes and stabilises the genetically specified synaptic organisation ("functional verification" hypothesis¹⁸). Dysfunction at critical periods of development may, however, lead to the regression of preformed, but already specified, synaptic connections¹⁸.

On the other hand, "empiricists" postulate that, to a large extent, the activity of the system specifies its connectivity, for instance by orienting the growth of the nerve terminals (see refs 17 and 19) or tracing pathways in more or less random networks^{20,21}. Third, as a compromise between these two attitudes, we have postulated^{22,23} ("selective stabilisation hypothesis"; see also refs 24 and 25) that the genetic program directs the proper interaction between main categories of neurones, for instance through the mechanisms presented below. However, during development within a given category, several contacts form at the same site; in other words, a significant but limited "redundancy" or fluctuation of the connectivity exists. The early activity of the circuits, spontaneous (in the embryo) and evoked (after birth) would increase the specificity or the order of the system, by reducing this transient redundancy. As a mechanism it was further postulated that the first synaptic contacts to form may exist under, at least, three states (synaptic plasticity): labile (L), stable (S) and regressed (D), the growth process being viewed as the emergence of labile states (Not→L). The labile and stable states would transmit nerve impulses but the regressed one (obviously) would not.

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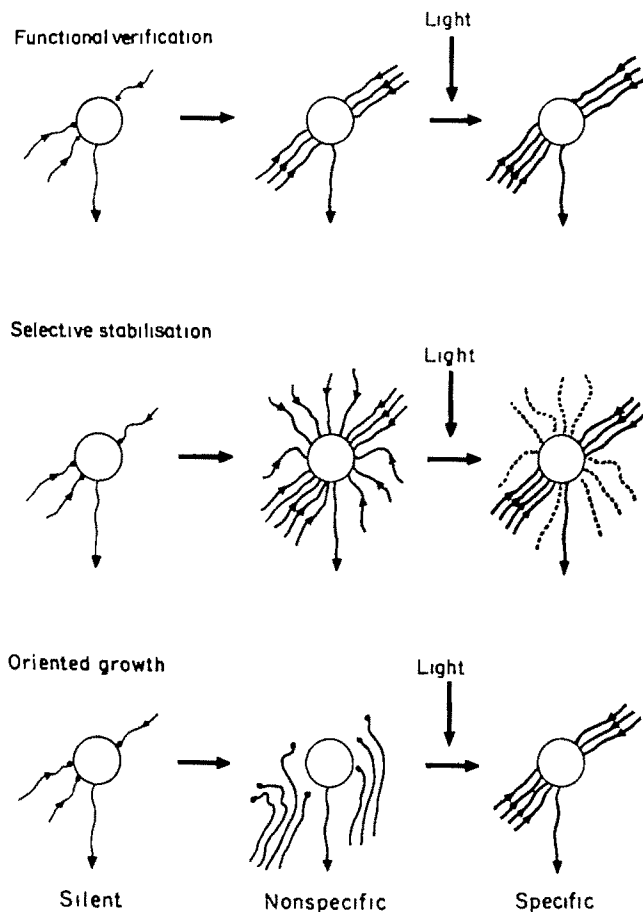


Fig. 1 Hypotheses regarding the effect of functional activity on the specification of a neuronal network: here a cell from the visual cortex which, in the adult, is specific for "orientation"¹⁸. According to Buisseret and Imbert²⁰¹, in the course of development the first active units to appear are "nonspecific" and respond to stimuli moving in any direction of the visual field. Subsequently, they give rise to "specific" units activated by light spots moving along only one precise orientation. The three possibilities considered deal with changes in the connectivity but alternative (or additional) mechanisms may take place such as changes of efficacy of excitatory and/or inhibitory synapses, growth of new sets of connections and so on.

The labile state may either become stabilised ($L \rightarrow S$) or regress ($L \rightarrow D$), regression being irreversible. (Regrowth ($Not \rightarrow L$) may however take place.) An essential statement of the theory²² is that the transitions of the labile state of the synapse ($L \rightarrow S$ and $L \rightarrow D$) are regulated, in an "epigenetic" manner, by the total activity of the postsynaptic cell, including the activity of the considered synapse. Accordingly, the precocious activity of the developing network would "stabilise selectively" particular synapses among the many equivalent contacts which emerge during growth and thereby create diversity and specificity (in a concomitant manner the non-stabilised contacts will regress). As a consequence, a critical period exists in the development of a nerve terminal when it requires a given pattern of activity to become stable.

This selective stabilisation hypothesis has been formalised and applied to a few systems²². One of its advantages is that it may afford an economy of genes. Indeed some of the genes which dictate, for instance, the general rules of growth, the stability properties of the immature synapses (postulate 1), the regulation of their stability by the activity of the immature synapse (postulate 2), the integrative properties of the postsynaptic neurone, may be shared by different categories of neurones or even be common to all neurones. The set of genes involved (the genetic envelope) should therefore be smaller than if each synapse were determined individually. The amplitude of the limited redundancy subjected to functional epigenesis may vary

from one area of the nervous system to another and with the "complexity" of the organism considered. Negligible in some invertebrates, it becomes most significant in high vertebrates.

In the following, we shall review experimental observations taken primarily from vertebrate neuromuscular junction and cerebellum which bring some support to the selective stabilisation hypothesis. Finally, we shall discuss biochemical mechanisms which may account for the stabilisation process and therefore make it more plausible.

Selectivity of surface recognition reconsidered

The exquisite specificity of synapse formation and its interpretation in terms of selective recognition between cell surfaces has often been emphasised¹¹⁻¹⁶ but functional synapses between non-homologous pairs of cells^{12,14,26-28} may form. In mammalian cerebellum the afferent mossy fibres normally contact granular cell dendrites. By various means²⁰⁻²⁸, the granular cells can be destroyed. In spite of the absence of their normal target, the mossy fibres make both anatomical^{29,38} and functional³³ synapses on another category of neurones: the spines of the Purkinje cell dendrites. Functional heterologous synapses have also been obtained in the frog between the vagus nerve and the skeletal sartorius muscle transplanted into the thoracic region^{39,40} and in the rabbit between the thoracic vagus and the diaphragm⁴¹; their occurrence has also been reported with the sympathetic ganglion after denervation⁴²⁻⁴⁵ or *in vitro*, in tissue culture^{46,47}, even between cells which under normal conditions do not synthesise complementary transmitter and receptor⁴⁸.

These few examples illustrate that, in the absence of the normal target or after deviation of the axons from their usual route, functional synapses may form between non-complementary partners as long as the receptor for the neurotransmitter is present in the postsynaptic cell. In these instances, at least, the selectivity of surface recognition does not appear as stringent as one would expect from the strict "chemo-affinity" hypothesis. Compatibility between cell surfaces rather than complementarity might suffice for a synapse to be formed and additional mechanisms have to be invoked.

Variations in adult connectivity

If the paradigm of a highly selective and genetically determined development of synaptic connections were absolutely correct, one would expect the anatomy of the adult nervous system to be identical in genetically identical individuals. To obviate the genetic heterogeneity of a natural population, organisms have been studied that reproduce parthenogenetically: a small crustacean (*Daphnia magna*)⁴⁹ or even a viviparous tropical fish (*Poecilia formosa*)⁵⁰. In a clone of such organisms, the different individuals have the same genetic equipment. The three-dimensional reconstruction of the fine structure of the optic system (eye and optic ganglia in *D. magna*) reveals that the gross features of the anatomy, in particular the number of cells, are invariant. A significant variability appears, however, in the branching and synaptic patterns of their processes at a resolution of a few tenths to a few micrometres. The variation seems to be as great when a given neurone is compared with the symmetrical cell on the opposite side of the same animal and with the same cell in other members of the clone. In spite of genetic homogeneity the "exact" form of identifiable neurones and of their processes shows a significant dispersion from one individual to another. The limits of genetic determination have been reached.

Activity of the growth cone and of developing nerve terminals

The primitive contacts present early in development transmit nerve impulses, long before the differentiated synapses of the adult are formed. In chick embryo, for example, spontaneous movements begin as early as 3 d of incubation⁵¹ and are of neurogenic origin⁵¹⁻⁵⁴. The rat foetus also moves spontaneously after 15 d of life⁵¹. At this precise date exploratory fibres enter the diaphragm and establish functional contacts (in 3 of 30

cells impaled) even before the presence of acetylcholinesterase can be detected by the Koelle method⁶⁵. One day later, however, it is claimed that a single localised spot of acetylcholinesterase is present in 30% of the myotubes and distributed at random on the surface of the myotubes (although there is no convincing demonstration of this last point). In the majority of the cells impaled evoked postsynaptic potentials are recorded. In chick slow muscle anterior latissimus dorsi (ALD) a similar event takes place except that several spots of cholinesterase are observed at regular intervals along the length of the developing muscle fibre and persist in the adult fibre⁶⁵. This post synaptic localisation process concerns both the esterase⁶⁶⁻⁶⁹ and the cholinergic receptor protein which, dispersed evenly on the surface of the developing myotube, soon become concentrated under the ingrowing nerve terminal⁶⁰⁻⁶². In tissue cultures the rudimentary synaptic contacts which form also transmit nerve impulses⁶³⁻⁶⁶. Even the growth cone or its filopodia show anatomical features indicating presynaptic or postsynaptic relationships with cell bodies or processes⁶⁷⁻⁷⁰ with local accumulation of vesicles or postsynaptic thickenings and may give rise to typical postsynaptic potentials⁷¹. Chemical transmission therefore exists days before the pre- and postsynaptic differentiations of the mature synapse become apparent.

Cinematographic films of neurones in tissue culture show that the growing processes^{72,73} are in perpetual motion. They advance, divide, retract, start again until a contact with a cell body becomes stable. It seems that through trial and error only a few of the transient contacts made by the growth cone or the developing neurites are selected. An interesting possibility is that such a behaviour accounts at least in part, for the fluctuation observed in the adult connectivity and that the activity of the growing processes play a role in this selection.

Spontaneous regressive phenomena

In the course of neurogenesis, regressive phenomena have been reported to occur at two distinct stages. (1) At the time of neuroblast "proliferation or immediately after, a significant fraction of neuronal somas die"⁷⁴⁻⁷⁸. For example, the ventral horns of the spinal cord of *Xenopus* tadpole may contain a maximum of 5,000 or 6,000 cells but 60 d later, at the time of metamorphosis, only 1,200, the adult number, persist. The number of cells which disappear during this period could even be larger (10,000) than the overall difference in total cell number⁷⁹. Similar events take place in the spinal cord of chick embryo⁷⁸ and foetal mouse (between days 10-15 of intra uterine life^{80,81}) and in several other parts of the nervous system (see ref 77). At later stages of development a second regressive phase affects primarily axon collaterals and dendritic branches without significant changes in cell number. For example, in foetal rat diaphragm, at d 17, soon after the dense acetylcholinesterase (and acetylcholine receptor) deposit appears in the middle of the muscle fibre, motor axons develop multiple branches and make a complex network which increases in size until birth⁸². Observation of the endplate by electron microscopy shows several synaptic profiles in contact with the same synaptic folds. The "complexity" of the endplate potential (its stepwise increase of amplitude as a function of stimulus strength⁸³) furthermore reveals that the several motor axons which converge on a given endplate are functional^{85,82-84}. During the first postnatal week, the number of steps of the complex endplate potential progressively decreases: one of the endings finally dominates the others. At 3-5 weeks, all the synapses are innervated by a single nerve terminal and non-innervated muscle fibres are not seen. More than 60% of the functional contacts have, therefore, disappeared. The fine structure of the endplate during the regression phase does not reveal any gross degeneration of axonal branches and the number of axons in the ventral roots does not change significantly⁸⁵. These observations have been repeated with other muscles⁸³, in particular with avian ALD⁸⁶ where each of the several endplates present per muscle fibre becomes innervated by several axon terminals.

Since the number of muscle fibres^{85,86} and of motor axons^{87,88,83} remains constant during this period, the number of muscle fibres innervated by a single motor neurone, that is the size of the "motor unit", should change^{88,89,83}. Indeed, during postnatal development the average size of the motor units decreases. In the 3-d-old rat soleus, a single motor unit tension is about 1/4 of the total muscle tension⁸³. In the adult, the fields of innervation of the motor neurones no longer overlap and a single motor fibre commands the contraction of a five times smaller set of fibres. This permits a progressive recruitment of a larger number of independent units and therefore a graded contraction of the muscle. The regressive process, which leads to the establishment of the "one motor terminal-one muscle fibre" relationship and the corollary decrease in size of the motor units, corresponds to an improvement of the motor command of the muscle. One may say that the selective elimination of redundant synapses increases the "specificity" of muscle innervation.

In the adult cerebellum, the majority (more than 90%) of the Purkinje cells receive only one climbing fibre^{17,90}. Unit recordings in 8-9-d-old rats show that, at this early stage, the response of the Purkinje cell to climbing fibre stimulation is "complex" as in the case of the immature neuromuscular junction: more than 50% of the Purkinje cells would be innervated by at least two distinct climbing fibres. A subsequent regressive phenomenon leads, as nearly as 15 d after birth, to the one-to-one relationship of the adult^{91,92}.

In these instances, the genetic program of the organism allows for an overproduction of labile and functional contacts but during maturation a significant fraction of them subsequently regresses.

Effects of target cell and its functional activity on synapse stabilisation

In the chick embryo⁹³⁻⁹⁶ and in amphibians⁹⁶⁻⁹⁸ early limb bud extirpation causes a massive depletion of both motor neurones and sensory ganglion cells accompanied by a decrease of the enzyme choline acetyltransferase in the spinal cord⁹⁹. Removal of the peripheral target cells dramatically enhances the "normally occurring cell death". An interaction with the periphery is therefore required for the neurone to continue its development.

In the mutant mouse "staggerer"³⁴, similarly, a massive regression of the parallel fibres and granular cells occurs at late stages of development¹⁰⁰ and has been attributed to the failure of parallel fibres to establish normal synapses with the Purkinje cells (which show electrophysiological signs of abnormality¹⁰¹ and lack a characteristic membrane protein¹⁰²).

In none of these instances, however, has evidence been presented that the actual activity of the postsynaptic cell has any effect on these regressive phenomena. To test this possibility, several cholinergic agents or toxins known to block selectively neuromuscular transmission were injected into developing chick embryos¹⁰³⁻¹¹². These drugs stop the spontaneous movements of the embryo which may, nevertheless, survive until hatching. Such treatment causes a marked atrophy of skeletal muscles which show signs of delayed differentiation and of regression¹⁰³⁻¹¹². Pre- and postsynaptic toxins have similar effects, which suggests that the actual "functioning" of the muscle by way of the synapse is necessary for its growth, histogenesis and maintenance. Interestingly, these compounds affect the innervation¹⁰⁷⁻¹⁰⁹. For example, a typical postsynaptic blocking agent, the α -toxin from the venom of *Naja nigricollis*, causes the almost complete absence of typical motor endplates as revealed by the Koelle reaction (the embryos were injected at 3, 8 and 12 d of incubation with high doses of α -toxin and observed at 16 d). The same happens with another postsynaptic antagonist: *d*-tubocurarine¹¹⁰⁻¹¹². In addition, and in a rather unexpected manner, despite the fact that α -toxin binds selectively to the nicotinic receptor site, marked regression takes place on the presynaptic side of the junction, as manifested by

a decrease in the specific and total activity of choline acetyltransferase in the muscle and sciatic nerve¹⁰⁷ and a reduction (more than 50%) in the total number (myelinated and non-myelinated) of axons in the ventral root of the spinal nerves (in the dorsal roots no significant change takes place)¹⁰⁸. A selective loss of the motor neurones takes place. This regression seems to be less significant with the presynaptic botulinum toxin at the concentration tested possibly because of an intrinsic effect of the toxin on the nerve terminal (for example it is known that botulinum toxin poisoning causes axonal sprouting at the adult neuromuscular junction¹¹³.) Although a direct action of the α -toxin on the neurones of the spinal cord^{114–115} cannot be ruled out in these experiments, no α -³H-toxin binding was detected in spinal cord extracts; in addition, the number of fibres counted in the spinal roots clearly shows that the effect of the α -toxin is limited to the cholinergic motor neurones¹⁰⁹.

How can an essentially postsynaptic block influence, then, the motor nerve terminal? The possibility that the α -toxin interferes with the "recognition" step at the early stages of synapse formation is made unlikely by the observation that primitive junctions (as well as the content of choline acetyltransferase) in embryos injected at the 4th d of incubation do not significantly differ from those of the non-injected control until the 12th d of incubation. Similarly, *d*-tubocurarine or α -bungarotoxin do not prevent synapse formation *in vitro*^{116–119} or re-innervation of adult rat diaphragm¹²⁰.

In agreement with this interpretation are recent findings on the effect of another snake α -toxin (from *Bungarus multicinctus*) on the development of retinotectal synapses in the toad: *Bufo marinus*¹²¹. The α -toxin was applied to a circumscribed region of tectal surface where it blocks the postsynaptic acetylcholine receptor in a stable manner for several weeks. In regeneration experiments, the optic nerve initially invades the toxin treated regions and subsequently retracts. In situations where the optic nerve was intact, chronic application of α -toxin results in a subsequent loss of optic nerve synaptic terminals. As in the case of the neuromuscular junction the stabilisation of developing nerve terminals requires the functional interaction of the neurotransmitter with its postsynaptic receptor.

Preliminary evidence suggests that the late phase of synapse maturation, that is the regression of multiple innervation, is also coupled with activity. Section of one of the tendons of sartorius muscle in infant rats indeed significantly delays this selective regression⁶⁴ but it is not yet known whether neonatal tenotomy modifies the chronic firing of the motor neurones. Also, in the cerebellum, as a consequence of the absence of the Purkinje cell major input (the lack of granular cells and their parallel fibres in X-irradiated rats^{122–123} and "weaver"¹²⁴ mouse), the multiple innervation by climbing fibres observed in the infant rats persists in adulthood.

Biochemical models for selective stabilisation

These few biological examples briefly reviewed provide some support for the "selective stabilisation" hypothesis. In particular, it is clear that (1) at critical stages of development neuronal bodies as well as growing neurites may exist under a functional labile state susceptible to death or regression (postulate 1); (2)

the number of neuroblasts and nerve connections produced at critical stages of development is significantly larger than that persisting in the adult (postulate 2); (3) the activity of the network may contribute in a direct or indirect manner, to this stabilisation process (postulate 3): in particular, retrograde signals emitted by the postsynaptic soma seem to have a significant role. Regarding this last point, the role of the "nerve growth factor" as a retrograde signal in the development of the sympathetic ganglion has already been extensively documented and reviewed^{125–127}.

The following discussion deals with biochemical processes, relevant to the mechanism of synapse stabilisation. We shall distinguish events taking place on the postsynaptic side of the synapse (primarily the "localisation" of the receptor protein) from those concerning the nerve terminal and its selective "maintenance" or stabilisation. The examples and reasonings presented concern the developing neuromuscular junction in vertebrates but might be adapted to more complex neuronal networks.

Postsynaptic 'localisation' of the receptor protein

This discussion deals with the 'localisation' of the acetylcholine receptor, but it should be borne in mind that localisation of acetylcholinesterase takes place simultaneously. In the adult neuromuscular junction the density of acetylcholine receptor sites counted with radioactive snake α -toxins is 100–1000 times higher than in extrasynaptic areas^{128–134}. It reaches an average of 8,000–9,000 sites per μm^2 (refs 128–132) in mouse junction with a higher density in the top (30,000 \pm 60,000 sites per μm^2) than in the bottom of the synaptic folds^{133,134} and up to 50,000 \pm 15,000 sites μm^2 in *Electrophorus* electroplaque (refs 135–137 and J. P. Bourgeois, J. L. Popot, A. Ryter and J. P. C., unpublished). The sub-synaptic membrane consists of a closely packed (lattice) assembly of the receptor molecules. Once formed the subsynaptic membrane appears remarkably stable: after denervation, the main features of the endplate region^{138–140}, the dense deposit of acetylcholinesterase revealed by the Koelle technique^{2,141} and the high sensitivity to iontophoretically applied acetylcholine^{142–144} persists for weeks. In *Electrophorus* electroplaque, the density of α -³H-toxin sites in the subsynaptic membrane decreases by less than 50%, 52 days after denervation (refs 136, 137 and J. P. Bourgeois, J. L. Popot, A. Ryter and J. P. C., unpublished). No significant tendency for lateral diffusion of the receptor protein exists either in the adult "uncovered" neuromuscular endplate^{145,146}. In the developing rat or chick myotubes, the density of acetylcholine receptor sites lies in between that of the extra and subsynaptic areas in the adult synapse (1,500/2,000 α -toxin sites per μm^2)^{147–149}. Although the actual translation mobility or the receptor protein in embryonic cells has not been measured yet, it should be close to that of the bulk proteins in a highly fluid cytoplasmic membrane^{150,151} that is much higher than in the subsynaptic membrane of the adult synapse.

The turnover rate of the cholinergic receptor protein in junctional and extra-junctional areas has been measured either with α -¹²⁵I-bungarotoxin (the particularly high stability of the toxin-receptor complex makes possible measurements of turnover rates faster than its own half life (9–15 d)^{148,152–154}) or directly after pulse labelling of the receptor protein by ³⁵S-methionine^{155,156} or heavy isotopes¹⁵⁷ followed by immunoprecipitation and/or purification. In developing calf myotubes *in vitro* the two methods give identical turnover rates¹⁵⁸. Table 1 shows that the degradation of the receptor protein occurs at a rate considerably faster in extra than in subsynaptic areas both *in vivo* and *in vitro* and the rate in the extrasynaptic areas of the adult muscle after denervation is close to that observed on the surface of the developing myotube. The degradation process is blocked by inhibition of oxidative phosphorylations: DNP, cyanide (possibly because of an energy-dependent "internalisation" process) but insensitive to inhibitors of protein synthesis (cycloheximide, puromycin)¹⁴⁸. The rate constant of

Table 1 Degradation of the cholinergic receptor protein

Extrasynaptic:	
developing myotubes <i>in vitro</i> :	
—decay of bound α -toxin	16–28 h (ref. 148)
—chase of ³⁵ S labelled receptor and decay of bound α -toxin	16 h (ref. 158)
adult rat diaphragm:	
<i>in vivo</i>	19 h (ref. 153)
<i>in vitro</i>	8–11 h (ref. 154)
Subsynaptic:	
adult rat diaphragm:	
<i>in vivo</i>	7–5 d or more (ref. 153)
<i>in vitro</i>	6 d or more (ref. 154)

degradation of the extrasynaptic receptor appears independent of the culture conditions and in particular does not vary with the growth rate of the developing myotubes (ref. 149 and J. Merlie, unpublished). Under these conditions the regulation of the number of receptor molecules therefore depends directly on its rate of synthesis.

Pharmacological differences have been reported *in vivo* between the subsynaptic and the extrasynaptic receptors^{60,61}; in addition, noise measurements reveal that the opening time of the cholinergic ionophore increases after denervation⁴; yet, they may not reveal differences in the primary structure of the two classes of receptor molecules but, for instance, differences in packing or environment of the receptor protein in the membrane. In agreement with this interpretation are the following observations. (1) *In vitro* the binding constants for agonists and antagonists of the membrane-bound receptor do not change in chick embryo leg muscles from 8 d (extrasynaptic) before hatching until 70 d after hatching (subsypaptic) (G. Giacobini, unpublished); the receptor proteins purified by affinity chromatography from extra and subsynaptic regions of rat diaphragm have been reported to show identical binding properties in one laboratory¹⁶¹ but different in another one¹⁶². (2) The equivalence point by immunoprecipitation seems to be identical for the two receptors (ref. 162, and H. Sugiyama, unpublished). (3) These two molecules have the same hydrodynamic properties¹⁶². Only isoelectric focusing unambiguously separates the two receptors, revealing a charge difference¹⁶³ but interconversion between the two structural forms has been obtained *in vitro*¹⁶³. The most likely interpretation of the data is that the extrasynaptic and subsynaptic receptors derive from the same protein molecule, the observed difference would result, for instance, from a covalent modification¹⁶⁴ but a different subunit composition of the two forms is not excluded.

Little is known yet about the factors which regulate the distribution and concentration of acetylcholine receptor in developing neuromuscular junction; however, the increase of sensitivity in extra-junctional areas after denervation is accounted for entirely by a neo-synthesis of receptor molecules^{155,165,166}. Direct electrical stimulation of adult denervated muscle through chronically implanted electrodes (refs 167–169 and T. Lømo, unpublished) or of embryonic non-innervated myotubes¹⁷⁰ maintained *in vitro* abolishes the hypersensitivity to ACh in the extra-junctional areas. The result depends critically on the amount and pattern of the stimuli. Maximal rate of decline of ACh sensitivity is approximately exponential with a half time in the range of that reported for the degradation in extra-synaptic areas^{168,169}. It is likely that optimal stimulation blocks receptor synthesis, the acetylcholine sensitivity falling at a rate

determined by the degradation of the extra-junctional receptor¹⁶⁹ (recent experiments¹⁷⁰ actually show that activity slows down this rate by about a factor of two).

To account for the localisation of the receptor protein and for eventual intercorrelations between developing synapses mediated by the postsynaptic cell we propose, that during development (Fig. 2):

(1) The receptor may exist under two interconvertible forms: *l*, labile and diffusible and *s*, stable, resistant to degradation and immobilised.

(2) The *s* state derives from the *l* state by way of a stabilisation reaction $l \rightarrow s$ which is not reversible. This reaction takes place when both an anterograde factor liberated by the nerve terminal during activity and an "internal coupling factor" are present above a critical concentration and within a given lapse of time, on the two faces of the subsynaptic membrane.

(3) On the membrane area underlying the developing nerve terminals, the neurotransmitter, in addition to its electrogenic effects, triggers the emission of a signal whose amplitude is given by the concentration of internal coupling factor which propagates inside the cell. (The propagation of the internal signal may result from a regenerative process. The initiation of the signal may take place with a significant delay in such a manner that its amplitude would reach the threshold for stabilisation only at a certain distance from the synapse. This would prevent, or delay, the self-stabilisation of the emitting synapse.)

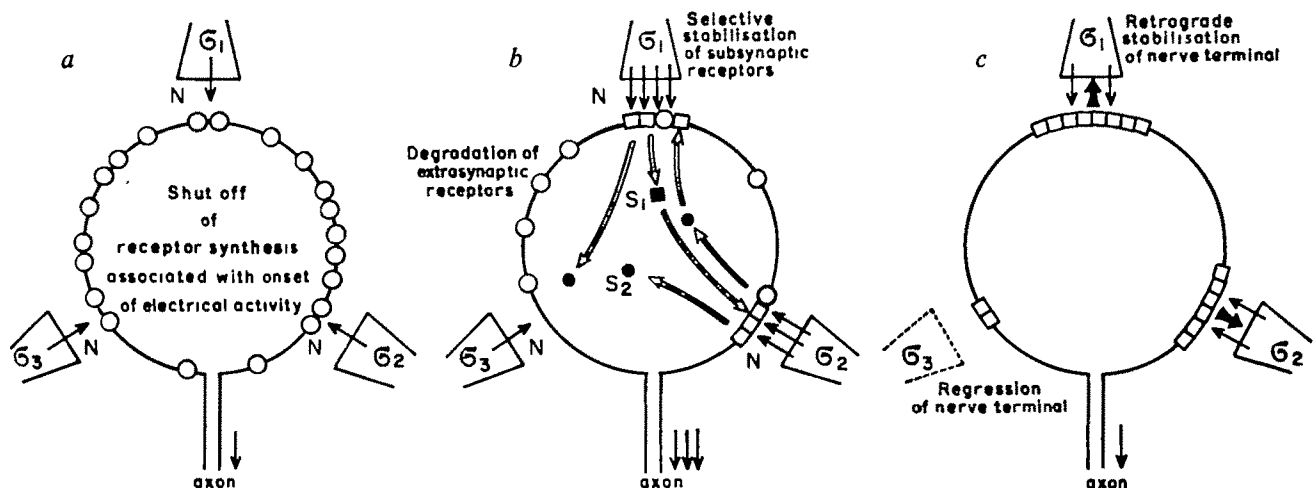
(4) The synthesis of the labile form of the receptor protein stops in the postsynaptic cell when activity starts in the myotube. An internal shut-off factor would act selectively on the protein synthesis machinery.

Accordingly, the selective localisation process would correspond to a management of a fixed and limited stock of *l* via lateral diffusion and stabilisation reactions.

This model is being formalised¹⁷² and may account for the redistribution of the receptor protein during localisation. The still hypothetical stabilisation reactions of the receptor molecule might for instance be a covalent modification of the receptor molecule such as a phosphorylation^{173–175}, an adenylation^{176,178}, a glycosylation^{177,178} or any other mechanism providing protection against proteolysis^{179,180}. The modification might for instance change the tendency of the receptor molecule to make aggregates under which it would be altogether resistant to degradation and immobile. It might also facilitate the anchoring of the receptor molecule inside the postsynaptic cell to fibrillar proteins¹⁸¹: actin, tubulin, or collagen, or, on its external surface to the basement membrane or any "cleft substance".

The most likely candidate for the "anterograde factor" is the neurotransmitter^{104,107,111,164}. This role in localisation has been

Fig. 2 The selective localisation of the receptor on the surface of a neurone receiving several nerve terminals. The labile and mobile state of the receptor for the neurotransmitter is represented by a circle, the stable and immobilised one by a square. The different signals postulated are represented by arrows: N, the anterograde factor, for instance the neurotransmitter; S, the internal coupling factor; a thick arrow indicates the retrograde factor. At the onset of activity a "shut off" factor stops the synthesis of labile receptor molecules. The model allows for the segregation of different receptor molecules if the nerve terminals secrete different anterograde factors.



challenged¹¹⁸ despite the fact that it provides a simple explanation for the segregation of different receptor molecules in a complex situation such as that of a neuronal soma receiving nerve terminals with different neurotransmitters. Other factors liberated by the nerve terminals such as, for instance, ATP^{188,189} or some of its degradation product enzymes or polypeptide hormones might have to be considered. The liberated ATP might offer a convenient energy supply for reactions taking place on the external surface of the membrane. Alternative (or additional) processes which do not require the emission of a diffusible signal might also have to be envisioned such as a "patching"^{181,182} of the receptor molecules in the postsynaptic membrane; spontaneous or resulting from a direct physical contact with the nerve terminal.

The postulation of one (or several) internal "coupling factor(s)", in addition to the membrane potential¹⁸⁴ is not necessary to account for the localisation of the receptor protein in the focally innervated muscle fibre. It may, however, offer plausible explanations for the development of complex patterns of synapses on neuronal somas (Fig. 2) or dendrites or, in the case of the avian ALD, of a regular distance between synapses. In any case it allows for interactions between synapses on a given soma in a more discriminative manner than the membrane potential. The postulated mechanism for the propagation of the signal is even more hypothetical than its very existence but plausible reactions may be written down which lead to the propagation of a "chemical" wave. (They might for instance be those of the nucleotide cyclase protein kinase and phosphatase system if, in one manner or another, the protein kinase target of the cyclic nucleotide exerts a positive feedback on the nucleotide cyclase.)

The "shut-off" of protein synthesis may be triggered by the internal coupling factor or by a different one. Possible candidates are the often evoked Ca^{2+} ions^{185,186}, cyclic nucleotides^{173,187} or both. Ca^{2+} would be a rather simple "shut-off" factor since it might enter through the cholinergic ionophore¹⁸⁸⁻¹⁹¹ or the tetrodotoxin sensitive ionic channels¹⁸²⁻¹⁸⁴ when they are activated. Signals directly coupled with the mechanical contraction of the muscle may also have a role.

In any case, despite its non-uniqueness the proposed model gives rise to several predictions which may offer experimental tests for the validity of its critical assumptions.

(1) If localisation corresponds to the management of a limited pool of receptor protein, changing the size of this pool by selectively blocking receptor synthesis before the arrival of the nerve terminals should interfere with localisation and/or reduce the number of endplates formed in a muscle like ALD. The "immunity" to innervation of the extrasynaptic areas in an adult muscle fibre would then simply result from the absence of receptor molecules available in the extrasynaptic membrane to form a novel endplate.

(2) The activity of the postsynaptic cell is expected to regulate the synthesis of receptor through the shut off factor but also the management of the membrane pool of receptor through the stabilisation reaction. Once the synthesis of receptor has stopped, changes in the program of activity of the developing nerve terminals by chronic stimulation (or blocking) may lead to the formation of supernumerary endplates (or prevent localisation) or, thanks to the internal coupling factor, for instance modify the distance between endplates. In avian ALD this may occur¹¹⁰⁻¹¹². In agreement with these views is the recent¹⁸⁶ observation that the spontaneous activity markedly differs in the slow ALD with multiple endplates and the focally innervated fast PLD from chick embryo (16-18-d-old): ALD fires continuously at a rate of 0.2 to 5 Hz, while in PLD only occasional activity is seen at a rate of 4 to 8 Hz. Chronic blocking of synaptic transmission may also modify the pattern of endplates and their relative distance in these muscles¹¹⁰⁻¹¹².

(3) According to the theory, the development of a characteristic geometric pattern of synapses may be determined by the exact temporal pattern of activity integrated by the postsynaptic cell. To illustrate this point, if Ca^{2+} is the shut off factor its

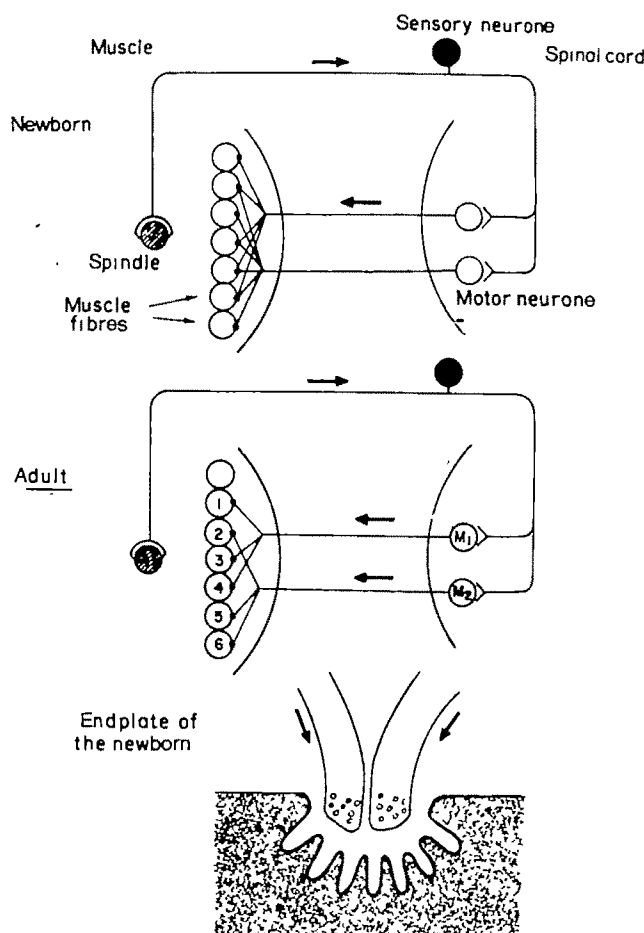
internal concentration might be regulated by two competing kinetic processes: the opening of the ionophores in the post-synaptic cell and the pumping rate of calcium by the mitochondria. A critical firing rate shall then be required to let the internal Ca^{2+} concentration reach a critical value to turn off the synthesis of receptor.

Presynaptic selection of a single nerve terminal at multiply-innervated endplates

Less information is available as yet on this second step of synapse maturation (Fig. 3), we may nevertheless propose that: (1) all the motor nerve terminals converging on a given endplate receive the same average number of impulses but in a randomly non-synchronous manner, (2) at the peak of the multi-innervation stage, all terminals share almost equivalent areas of the stabilised subsynaptic membrane surface and/or dispose of a limited stock of a postsynaptic "retrograde factor" x , (3) the arrival of impulses cause an increase of surface occupancy and/or of utilisation of x , (4) when the surface occupied by the nerve terminal (and/or the amount of x utilised) becomes lower than a critical value this nerve terminal regresses.

This set of minimal assumptions may explain both the stabilisation of a single nerve terminal and the constant size of the motor units. Accordingly, the size of the motor unit should be determined by the ratio of the number of muscle fibres to that of motor neurones. Indeed, it is known from human patients that a partial neurogenic atrophy (which reduces the number of motor neurones) causes a decrease in the number

Fig. 3 The differentiation of motor units in a skeletal fast muscle by retrograde selective stabilisation of nerve terminals. In the neonate one motor neurone innervates many more muscle fibres than in the adult and each muscle fibre receives several nerve terminals. A few weeks after birth only one nerve terminal persists per muscle, the others regress.



of motor units with a marked increase in their size¹⁹⁸⁻¹⁹⁹. An increase of surface occupancy by nerve terminals as a consequence of activity has been described^{199, 200}, but the biochemical mechanisms by which a nerve terminal becomes stabilised once a critical surface of the postsynaptic membrane is occupied remains obscure. The postulated retrograde factor (substance x) has not been identified chemically; in the case of the neuromuscular junction: the concentration of calcium in the cleft¹⁰⁸ or of any nerve growth factor are possible candidates. An interesting possibility would be the direct covalent modification of the fibrillar or tubular proteins engaged in the maintenance of the shape of the nerve terminal and thereby regulating its internal "cytoplasmic flow".

Conclusions

From a biochemical point of view, the selective stabilisation hypothesis appears plausible. Four different signalling mechanisms have been postulated to govern the evolution of a developing synapse: (1) for the postsynaptic localisation of the receptor protein: an anterograde factor liberated by the nerve terminals (possibly the transmitter) which governs the stabilisation reaction of the receptor, an internal shut off signal which informs the protein biosynthesis machinery (transcription and/or translation) of the activity of the postsynaptic cell, an internal coupling factor which permits exchange of information between synapses and 2) for the selective presynaptic stabilisation of the nerve terminals, a retrograde factor emitted by the postsynaptic cell. Additional mechanisms and regulatory signals may have to be postulated in the future to account for the complete description of the stabilisation and maturation of the synapse which is certainly a complex process lasting for days or even weeks. In any case, at this level of understanding the word "trophic factor" is of no help and should be abandoned.

The basic assumption of the theory that an increase of "specificity" accompanies the active stabilisation of particular synapses (and the regression of others) remains to be tested with neuronal networks (cerebellum, visual cortex) more complex than the motor innervation of the striated muscle. For instance, an intriguing possibility would be that in the much studied visual cortex (Fig. 1) the acquisition of a given specificity such as the specificity of orientation¹⁸, results from such a selective stabilisation (Fig. 1). It would be of primary interest to investigate through intracellular recordings if the transition from "nonspecific units"²⁰¹ to "specific" ones would correspond to a decrease of synapse redundancy via the regression of transiently multi-innervated neuronal somas and to what extent the activity of the system, spontaneous and/or evoked, governs these transitions.

In a more general manner, these speculations place emphasis on critical features of developing neuronal networks which are directly accessible to the experiment: (1) their early activity (spontaneous or evoked) preceding synapse maturation: (2) the existence and limits of a transient redundancy (in the case of motor innervation the limits of the fluctuation are the boundaries of the muscle²⁰²); (3) an eventual modification of this redundancy by chronic changes of the early activity.

Even if the limits of the redundancy are narrow, they may be sufficient to provide an opportunity to save genes during the differentiation of neuronal networks and to provide a plausible biochemical mechanism for "learning" without postulating any synthesis of new molecular species.

Many theoretical aspects of this paper result from work done in close collaboration with Philippe Courrège.

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articles

Ophiolites in south-western Newfoundland

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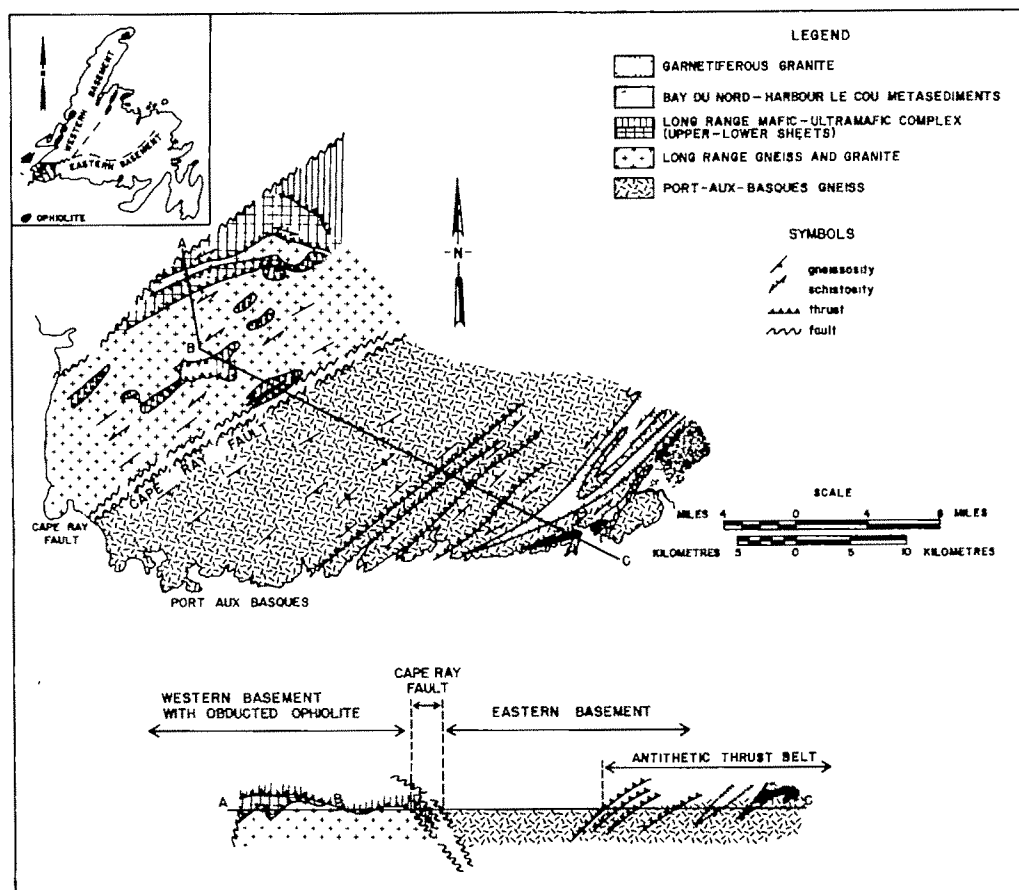
Ophiolite and ophiolite-related rocks have long been recognised in northern and western Newfoundland^{1,2}. The presence of these rocks has, to a great extent, controlled the plate tectonic models developed for the tectonic evolution of the Newfoundland Appalachians. Such models have been extrapolated through south and south central Newfoundland with little geologic control. I describe here the ophiolites in south-western Newfoundland, discuss their relationship to a cryptic suture, the Cape Ray Fault, and evolve an independent model, which I compare with the model developed for northern and western Newfoundland.

SOUTH-WESTERN Newfoundland consists essentially of two basement complexes separated by a zone of intense mylonitisation, the Cape Ray Fault. The Long Range Gneiss, to the North of the fault, forms part of the Western Crystalline Belt³ and, as such, defines the western margin of the Proto-Atlantic Ocean. The Port aux Basques Gneiss, to the South of the fault, forms part of the Eastern Crystalline Belt⁴ and defines the eastern margin of the Proto-Atlantic Ocean. The Cape Ray Fault has been interpreted as a cryptic suture, along which complete closure of the Proto-Atlantic Ocean took place⁴.

Lower sheet

Recent work (P. A. Brown, unpublished) in south-western Newfoundland has shown that the Long Range Mafic-Ultramafic Complex⁵ occurs as thrust sheets overlying a

Fig. 1 General geology of southwestern Newfoundland showing the relationship of the obducted ophiolite to the Cape Ray Suture and antithetic thrust belt.



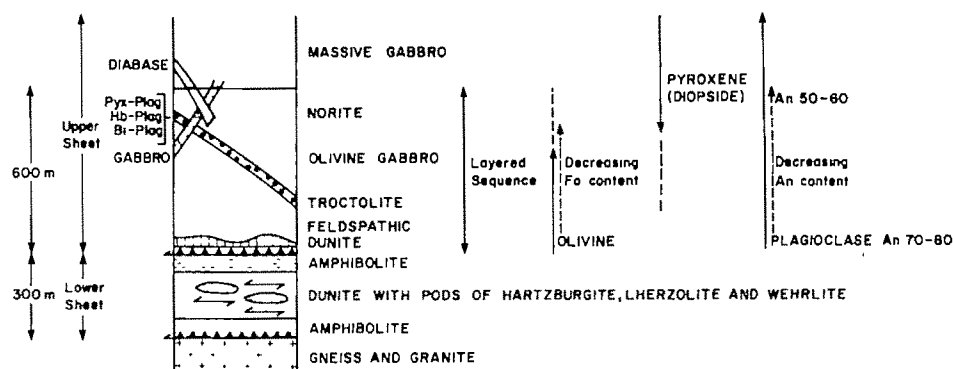
granitic gneiss terrane, the Long Range Gneiss (Fig. 1). There are two main thrust sheets, a lower, intensely tectonised sheet, and an upper, well preserved, less tectonised sheet (Fig. 2). The lower, dominantly ultramafic, thrust sheet consists of variably tectonised dunite, harzburgite, lherzolite and wehrlite. The harzburgites, lherzolites and wehrlites occur as tectonic 'pods' within highly serpentinised dunite. Original igneous textures are not preserved. There is an increase in deformation towards both the upper and lower contact of the sheet. The lower contact is defined by a highly schistose cummingtonite-pargasite-pyroxene schist. This schist is in tectonic contact with granite and granite gneiss of the basement and is interpreted as the basal thrust of the mafic-ultramafic complex. Locally this contact is marked by a serpentinite mélange, with blocks of serpentinite up to 50 cm in diameter set in a carbonate matrix. The upper contact is defined by a well-developed actinolite-zoisite schist which grades upwards into a locally thick sequence of well banded amphibolites. The banding is discontinuous and irregular and is igneous in origin. The amphibole characteristically shows a blue-

light green pleochroic scheme and is probably pargasitic in composition.

Upper sheet

The upper sheet, in contrast, is well preserved, little tectonised, and a lithological sequence can be outlined. Within the sheet there is a decrease in tectonism away from the Cape Ray Fault. The rock types vary from feldspathic dunite at the base, through a well layered sequence of troctolite, olivine gabbro, norite and anorthosite, and finally into massive, coarse to fine grained, gabbro at the top (Fig. 2). Two types of layering are present. A rhythmic layering of width 5-20 cm with olivine- or pyroxene-rich bases and plagioclase-rich tops. These show graded (mineralogical) bedding and slump features. The succession is nowhere observed to be inverted. At the base of the succession, olivine is the dominant mafic mineral. The percentage of olivine decreases upwards, and towards the top of the succession pyroxene becomes the dominant mafic mineral (Fig. 2). The second type of layering is cryptic and occurs on both a small and large scale. On a small scale, the composi-

Fig. 2 Generalised section of the Long Range Mafic-Ultramafic Complex.



tion of plagioclase shows a decreasing Anorthite content from the intercumulus crystals at the base to the cumulus crystals at the top of each band. On a large scale the plagioclase shows an overall change in composition from An_{70-80} at the base of the succession to An_{50-60} at the top. Olivine varies from $For_{90}Fa_{10}$ (cumulus) at the base to $For_{80}Fa_{20}$ (intercumulus) towards the top. The pyroxene appears to become more diopsidic towards the top. Dark green spinel (hercynite?) is found throughout the sequence.

Boundary regions

The boundary between the layered sequence and the massive gabbros is often marked by plagioclase-pyroxene pegmatites. The gabbro becomes finer grained upwards and is intruded by stringers and pods of a plagioclase-rich, potassium-feldspar-poor biotite granite. The entire sequence is cross cut by several types and ages of dykes. Pegmatitic pyroxene-plagioclase, hornblende-plagioclase, and biotite-plagioclase dykes cut the layered sequence. These are cross cut by gabbro dykes, which in turn are cross cut by diabase dykes with chilled margins.

The complex was emplaced along a series of thrusts that define a schuppen-type structure. There are two major thrusts. The lower (basal) thrust separates the lower sheet from the underlying granite and gneiss and most of the initial movement has taken place along this thrust. The upper (intraformational) thrust separates the lower from the upper sheet and results in a structural stacking of the mafic-ultramafic complex.

The Cape Ray Fault

To the south-east, the complex is bounded by a south-eastwards dipping mylonite zone, the Cape Ray Fault. The upper sheet becomes progressively tectonised towards this mylonite zone and, within it, is altered to a chlorite-actinolite schist. Most of the mylonite is derived from the granitic-gneissic basements that define the eastern and western margins of the Proto-Atlantic Ocean. This led to the Cape Ray Fault being interpreted as a cryptic suture along which complete closure of the Proto-Atlantic Ocean took place⁴.

In northern and western Newfoundland, the mafic-ultramafic rocks preserved as slices thrust onto the western crystalline belt (White Hills Peridotite and Bay of Islands Complex) have been shown to be ophiolite in origin^{1,6,7}. It is suggested that the similar setting (Fig. 1) of the Long Range Mafic-Ultramafic Complex indicates that this body is also of ophiolitic origin. This suggestion is supported by the similarity of the stratigraphy of the complex to that of the critical zone and upwards of many recognised ophiolites⁸ and specifically to that of the Bay of Islands Complex^{2,7}. Furthermore, the mode of emplacement, that is, obduction tectonics, is compatible with subduction-obduction tectonics. The presence of the complex only to the North-west and partially within the southwards-dipping Cape Ray Suture strengthens this interpretation and also indicates that the subduction zone must have dipped, at least in the final stages, to the Southeast.

The formation of the Cape Ray Suture implies an inter-continental continent-continent collision in south-western Newfoundland. It is likely that such collisions develop an antithetic thrust belt on the down dip side of the subduction zone⁹. Such a belt, involving high grade, shear zones, dipping to the north-west, and recumbent folds incorporating both basement and cover rocks, is found 20 km–30 km to the south-east of the Cape Ray Fault. The sense of movement is overthrusting to the south-east. Involved in this thrusting are garnetiferous leucocratic granites (products of extreme differentiation of calc-alkalic magmas¹⁰), which may be related to southeastwards subduction under the eastern crystalline belt.

The model proposed for the geological development of

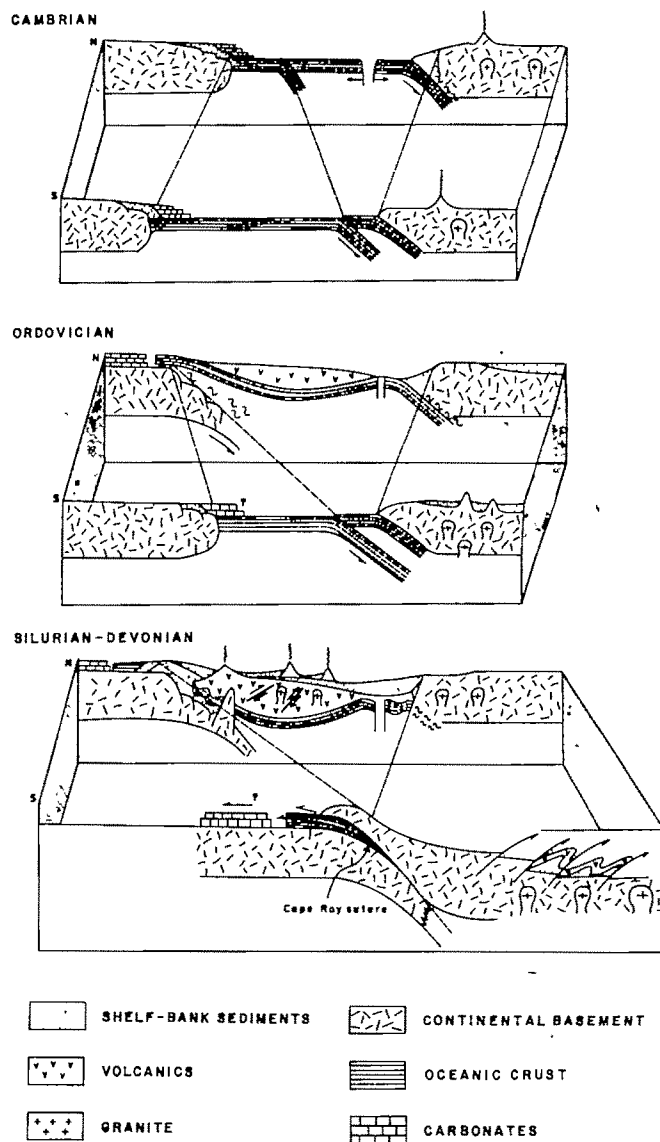


Fig. 3 Model for the closure of the Proto-Atlantic Ocean in Newfoundland. Northern sections after ref. 17.

this area is therefore:

(1) Southeastwards subduction under the eastern margin of the Proto-Atlantic resulting in calc-alkalic magmas and related intrusive differentiates.

(2) Closure of the ocean along the trace of the Cape Ray Fault with accompanying obduction of ophiolite onto the western margin and the development of an antithetic thrust belt.

(3) Final overthrusting of the western margin by the eastern margin resulting in internal thrusting and structural stacking of the ophiolite. The cessation of thrusting is probably related to the isostatic upward pressure of the downthrust western continental slab.

Although this model is, in general, similar to recent models developed in northern Newfoundland, there are significant differences: first, the obducted ophiolites to the north and west occur as large slabs thrust onto shelf-bank sediments and these sediments are themselves involved in the thrusting. In south-western Newfoundland the ophiolite is dismembered and is thrust directly on to a gneiss and granite basement. Second, the timing of final emplacement in the north is Middle Ordovician^{12,13}. In the Southwest the obduction event cannot, at present, be dated, but the related antithetic thrust belt affects cover rocks (the Bay du Nord Group) which contains Lower Devonian fossils¹⁴.

The different style of ophiolite emplacement in northern and southern Newfoundland suggests either that the Cape Ray Fault is a deep level exposure of the obduction event in the North (see Fig 4, ref. 15) or that there are two independent styles of obduction. The first possibility is attractive in that it explains the lack of shelf-bank sediments and the dismemberment of the ophiolite in the southwest. It fails, however, to explain the development of an antithetic thrust belt of Acadian age if complete closure took place in the Middle Ordovician. Furthermore, the calc-alkalic garnetiferous granite involved in the antithetic thrusting has been dated at 415 ± 15 Myr (R. Cormier, personal communication), indicating that subduction continued under the eastern continent in post Middle Ordovician times.

The second alternative may be related to the early development of the system. In northern Newfoundland, an early Pre-Middle Ordovician sequence of island arc volcanics¹⁴ was developed above a subduction zone^{11,17} dipping to the east. These volcanics thin the South-west and, in central Newfoundland, both island-arc volcanics and garnetiferous leucocratic granites are present (B. F. Kean, unpublished). Further to the Southwest, the volcanics disappear along the trace of the Cape Ray Suture. Garnetiferous leucocratic granites are, however, present in southwestern Newfoundland.

The absence of the volcanics indicates either that they were obducted along the suture or else they were never developed in south-western Newfoundland. If they were removed by obduction there should be a great increase in intensity of deformation towards the suture. This does not occur (B. F. Kean, unpublished). Thus the volcanics were never developed in southwestern Newfoundland. Subduction did, however, occur in this area, as indicated by the presence of garnetiferous leucocratic granites to the South-east of the suture.

This southwards thinning of the island arc volcanics and incoming of garnetiferous leucocratic granites can best be explained by an asymmetric subduction event, that is, the subduction zone was not parallel to the continental margin. In northern Newfoundland subduction occurred dominantly under oceanic crust. In central Newfoundland subduction

occurred under both oceanic and continental crust. In southeastern Newfoundland subduction occurred dominantly under continental crust (Fig. 3).

In the North, the obduction event was related to a continent-oceanic crust island arc collision around Middle Ordovician times. Minor subduction continued through Silurian times, however, with the development of a late arc sequence¹⁸ of dominantly calc-alkaline volcanics. This sequence thins to the South-west and disappears along the trace of the Cape Ray Suture. In the South, continued subduction of oceanic crust from Pre-Middle Ordovician times to Late Silurian-Early Devonian times resulted eventually in the complete closure, that is, continent-continent collision, of the system. This caused ophiolite obduction, the overriding of the eastern over the western continent, the dismemberment of the ophiolite, and the development of an antithetic thrust belt.

Thus the closure of the Proto-Atlantic Ocean and the variable styles and ages of ophiolite obduction are seen as a complex sequence of events controlled by, and related to, the asymmetry of subduction with respect to the continental margins.

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Re-analysis of radiation-induced specific locus mutations in the mouse

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A re-analysis of published data on mouse mutation rates induced by X and gamma rays suggests that the kinetics of induction can be analysed by fitting the data to a parabolic curve. We interpret this to mean that a substantial proportion of the induced mutations results from gross chromosomal changes such as deletions, some of which are one-track and some of which are two-track. This analysis is based on the assumption that the shape of the dose curve, which in the female is concave upward, reflects the manner in which the mutations are induced rather than representing a one-track (linear) curve whose shape has been modified by differential repair.

MOST of the data dealing with the genetic effects of ionising radiation in mice have been obtained with a specific locus test. The data were obtained under many different experimental conditions¹⁻¹⁷ (see refs 18 and 19 for review), and form a major element in the evaluation of possible genetic effects of radiation in man^{19,20}.

The mutations have commonly been considered to be one-track events that increase linearly with dose, although the statistical errors associated with the data are so large that they can be fitted by non-linear curves²¹. Furthermore, the mutagenic efficiency of the radiation has varied with the experimental conditions: densely ionising radiations have a high relative biological effect, or RBE⁶, and sparsely ionising radiations can be less efficient if the dose is protracted over long periods or fractionated into sub-doses given at various intervals. These constitute criteria by which true point

mutations can be distinguished from two-break chromosomal effects, and it was postulated that many (if not most) of the specific locus mutations induced by X rays are two-track deletions²¹. Many of these specific locus mutations have indeed subsequently been shown to be deletions affecting more than one functional unit²². These deletions, however, have been considered to be one-track events⁸ even though the dose-effect curve obtained by irradiating female mice with acute doses of X rays is concave upwards^{8,14}.

In order to account for dose protraction and fractionation effects, the concavity of the curve obtained from females, and so on, various mechanisms have been postulated. These have included dose-dependent intracellular repair of a one-hit phenomenon⁸, cell selection²³, and cell synchronisation³.

Rather than treating an induction curve for mutations that is concave upwards as if it represented a one-track phenomenon that empirically did not increase linearly with dose, we believe that it is fruitful to treat the specific locus mutations as if they were a mixture of two-track²¹ and one-track²² events, mainly deletions.

We have fitted the data on mouse-specific locus mutations induced by X rays that were presented in Searle¹⁸ and the UNSCEAR report¹⁹, as well as more recently published data^{13,14,17}, to the curve represented by the quadratic equation,

$$Y = C + \alpha D + \beta D^2$$

where Y equals the expected yield of mutations, C equals the control rate or spontaneous yield, α equals the coefficient of induction of mutations that occur as a linear function of the dose (D), and β equals the coefficient of induction of mutations that increase as the square of the dose. This equation has been used for the induction of two-break chromosome aberrations where two separate events are required. The events may be caused by a single ionising track (linear term) or by the interaction of two independent tracks (dose-squared term), that is, the same situation we postulate for specific locus mutations.

We believe that, in many cases, with the use of this equation it is possible to make quantitative estimates of the yields of mutations that would be obtained under different regimes of irradiation. For instance curves for acute exposures will include both linear and

quadratic components, but those for chronic exposures will be linear because the chance of interaction of independent events will be negligible.

Data from irradiated oocytes

For oocytes (Table 1), the data that determine the dose-effect curve, that is, the control point and the data obtained after single acute doses of 50, 200, and 400 R, were used to compute the regression equation. Each of the four points was weighted by the reciprocal of the binomial variance and the coefficients determined by least squares analysis. All the oocyte data in Table 1 came from matings occurring within the first 7 weeks of irradiation, since Russell¹⁰ has observed that the mutation rate derived from conceptions occurring after 7 weeks is near zero for single acute exposures. Only one litter was recoverable from the 400 R series, two litters from the 200 R series and three litters (within the 7 week period) from the 50 R series.

For the female data, three different expected values are presented. These depend upon the value for the control rate used in the analysis. Out of 164,999 unirradiated gametes tested in the same laboratory in which the 50, 200, and 400 R data were obtained, there were seven mutants observed. Six of the seven mutants, however, were offspring of the same female and represented a cluster of mutant germ cells that came from a single mutation that had occurred early in development¹⁰. Russell¹⁰ has pointed out that this cluster complicates the computation of the spontaneous mutation rate in females, and he has calculated two different rates. In one case, he assumed that the chance for mutation occurring in early development, when there was a limited number of germ cells, is less than the chance for occurrence at a later time when there are many more germ cells. Under this assumption, one would expect clusters to be much rarer than single mutations and there would be little error in assuming that only two mutations were obtained. In the other case, the assumption made was that the only estimate of the frequency of clusters was that observed in the data. This leads to the use of all seven recovered mutations in computing the spontaneous mutation frequency.

Although an estimate of the spontaneous rate in females could be based on seven mutants, this estimate, for reasons first pointed out by Luria and Delbruck²⁴, would have a very large error

Table 1 Specific locus mutations from irradiated mouse oocytes

Item	Ref.	Experimental conditions	Gametes tested	Observed	Number of Mutants Expected		
					Cluster = 0	Cluster = 1	Cluster = 6
1	10,18	Control	164,999	1,2, or 7	0.97	1.93	6.6
2	10,18	50 R Acute 250 KVP X ray	180,472	13 (6.7-21.4)	13.56	13.63	13.95
	5,18	200 R Acute 250 KVP X ray	37,297	21 (12.8-31.7)	19.07	18.85	17.75
	8,18	400 R Acute 250 KVP X ray	14,842	23 (14.9-34.0)	24.13	24.27	24.91
3	8,18	400 R Acute 250 KVP X ray (8 fractions of 50 R, 75-min intervals)	27,906	19 (11.2-29.0)	15.63	16.86	9.4
4	5,18	400 R Acute X rays (2 fractions of 200 R, 1-d interval)	6,086	9 (4.5-16.8)	6.19	6.15	5.55
5	4,18	400 R ¹³⁷ Cs (8 h. chronic αD contribution only)	20,827	7 (3.3-13.8)	8.49	7.57	3.02
6	11,18	600 R ⁶⁰ Co (12 d chronic αD contribution only)	10,117	1 (0.05-5.3)	6.16 (3.1)	5.45 (2.8)	2.0 (1.27)
7	10,18	258 R ¹³⁷ Cs (20 d chronic αD contribution only)	8,373	1 (0.05-5.3)	2.22 (1.13)	2.0 (1.0)	0.9 (0.62)
8	10,18	400 R ¹³⁷ Cs (31 d chronic αD contribution only)	29,325	3 (0.82-8.1)	11.91 (3.28)	10.69 (3.08)	4.27 (2.00)
9	14	200 R Acute X rays ($\alpha + \beta$, three litters, half of matings immediately after irradiation, half 1 week later)	34,813	9 (4.5-16.8)	17.78	17.66	16.59

The regression analyses were calculated when the spontaneous cluster was counted as contributing 0, 1, or 6 mutations to the control and is based on the four data points in items 1 and 2. These regression coefficients were used to predict the values in items 3-9. The numbers in parentheses are the expected values for the estimated effective dose (see text) delivered to maturing oocytes assuming that some of the irradiation was received by cells in insensitive stages. The numbers in brackets are the 95% confidence limits for the observed manner of mutations⁴⁴. Ref. 18 is a comprehensive review.

Table 2 Mutation coefficients calculated from data from spermatogonia and oocytes

	Male		Female Cluster counted as	
		0	1	6
C	8.30×10^{-6}	8.39×10^{-7}	1.67×10^{-6}	5.74×10^{-6}
α	6.88×10^{-8}	1.43×10^{-7}	1.26×10^{-7}	3.76×10^{-8}
β	6.57×10^{-10}	1.09×10^{-9}	1.14×10^{-9}	1.37×10^{-9}
α/β	104 R	131 R	111 R	275 R

α expressed as mutations per locus R^{-1} , β expressed as mutations per locus R^{-2} , C is the control frequency, α/β equals the dose where the linear contribution (αD) equals the two-hit contribution (βD^2). To fit the coefficients to the data presented in Tables 2 and 3, multiply the coefficients by seven, the number of loci examined

associated with it. Another way of treating the spontaneous rate is to make use of the fact that any cluster has to arise in pre-oocytic germ-cell stages. Since only oocytes were irradiated in the treated series, we are really concerned in our analysis only with spontaneous mutations arising in such cells. At most, one spontaneous mutation arose in oocytes sampled in the control series. As far as the treated series are concerned, no clusters were reported. Of course, single mutational events may also have arisen in oogonia, but this is true for both the irradiated and control series. For these reasons we believe that, in the present context, the best estimate of the control rate is based on only one mutant ignoring the cluster of six.

In Table 1 we present expected values based on all three estimates of the control rate.

The coefficients for the equation are given in Table 2 for all three fits. From these coefficients the expected number of mutants for other experiments was calculated. For items 3 and 4 (fractionated doses) of Table 1, the equation was used to determine the expected value for each component of the treatment and the effects were then added. For items 5–8, where the treatment was chronic (a dose rate of less than 1 R min^{-1}), only the linear component ($\beta=0$) was used, since the linear term should be independent of any dose rate effect. The agreement between predicted values and observed values when the cluster was either disregarded or counted as one mutation is excellent except for the last three items where, we believe, the period of irradiation was so long that the recovered oocytes were to a large extent irradiated in a variety of developmental stages including the most immature and less mutable ones (stages 2, and probably 3 of Pedersen²⁵).

Although many mouse strains have a 4–6-d oestrous cycle in which oocytes routinely mature and ovulation occurs, this can be readily modified by both endogenous and exogenous factors²⁶. For instance, the maturation of oocytes can be affected by pregnancy and by crowding the mice into all-female groups free from the scent of males (which suppresses oestrous) as well as by radiation (which induces superovulation²⁸ that can last for up to 16 d during which even immature oocytes can be ovulated²⁹). Thus, when radiation is administered over a prolonged period and the animals subsequently mated for 7 weeks, it is difficult to determine how much dose was accumulated in any given stage of oocyte development.

Precise knowledge of the stage of the oocyte at the time of irradiation is important because (1) the radiosensitivity varies with the stage and (2) oocytes sampled after acute exposure are different from those sampled after chronic exposure.

As regards the first, primordial oocytes (stage 2 of Pedersen) are killed far more readily by acutely delivered X rays than are mature oocytes³⁰. Paradoxically these primordial oocytes are the ones reported to have a near zero induced mutation rate with either low or high linear energy transfer (neutron) radiation delivered either acutely or chronically¹⁰. The reasons for the mutational insensitivity of these immature oocytes are obscure, although Russell has listed three possible explanations: (1) the immature oocytes are resistant to radiation damage, (2) their repair capacity

is greater, (3) the mutant cells are selectively eliminated. He favours the view that they have a greater capacity for repair. We note, however, that chronically irradiated female mice produce 60–70 offspring throughout their reproductive lifespan³¹. There are many thousands of immature oocytes in the ovary and over 4,000 are normally committed to further development, but not necessarily ovulation²⁵. Thus, about 98% of the cells are in some way eliminated, leading us to believe that selection against the mutants occurs.

As regards the sampling of different oocytes after acute and chronic exposures, we note that after acute exposures, in which the radiation was delivered in a few minutes, the majority of the matings that produced the first litters occurred within a few days of irradiation. Thus, the first litters came from oocytes that were in the most mature, and presumably the most mutable, stages. The second litters presumably came from oocytes that were in less mature stages. The third litters, which were produced only in the 50 R series, should have come from oocytes that were immature at the time of treatment^{25,27}. Unfortunately the data for the separate litters are not published, but it is likely that our mutation rate estimates after acute exposures are somewhat reduced because of the non-uniformity of the stages sampled.

In contrast, during chronic exposures lasting 12–35 d, the females may have had as many as nine oestrous cycles intervening between the inception of irradiation and the time of mating. Clearly then the oocytes providing the first, and particularly any subsequent, litters in the chronic series were in a different stage for most of the irradiation than their counterparts in the acute series.

Thus for all these aforementioned reasons, we think it is likely that the small numbers of mutants observed in items 6–8 are the consequence of some of the oocytes having been irradiated during the less mutable stages rather than resulting from repair of pre-mutational damage in a static cell population. The effective dose received by sensitive stages would then be less than the total dose delivered. We have attempted to assess the effective dose resulting from protracted irradiation on the simplifying assumption that there were ongoing oestrous cycles at 4-d intervals during the exposure. Thus if, as in item 6, the irradiation is protracted over 12 d, which is the transit time from stage 3b to ovulation²⁵, most of the cells recovered from a mating occurring immediately after the irradiation would have been mutation-insensitive immature cells at the beginning of irradiation. Approximately 4 d after the irradiation was initiated these cells would have moved into the maturing oocyte compartment to replace those which had ovulated. Their effective dose then would have been 8/12 of the nominal dose delivered. In this experiment, however, while most of the matings took place within 3d of the irradiation, some of the females in fact may have been mated over a period of 2 weeks¹¹, and several more oestrous cycles may have occurred before fertilisation. The consequence of this would be to provide oocytes that received only 4/12 to 0/12 of the dose while they were in stages 4–7 (the fraction would depend on the number of intervening ovulations). A conservative assumption would be that the progeny examined (all first litters) came from oocytes that received between 8/12 and 4/12, or an average of 6/12, of the nominal dose. This should lead to an expected number of 2.8 mutants for this experiment, or to an even smaller number if there were more ovulations or superovulations intervening between irradiation and the mating. Similar calculations were made for items 7 and 8, and the expected numbers appear in parentheses in Table 1.

These crude corrections bring the expected numbers into reasonable agreement with the data. We do not argue that these corrections are appropriate, but our present ignorance of the effects of irradiation on the early oocyte stages does not justify a more elaborate treatment. For the present, however, we believe that it is reasonable to assume that the discrepancy in items 6–8, where the irradiation extended over many days, is caused by some of the radiation occurring during less mutable stages.

Item 9 shows recent data from another laboratory¹⁴. Seven of the nine mutants appeared in the first litter and only two in the second. We have no specific explanation why the yield here is

Table 3 Specific locus mutations from irradiated mouse spermatogonia

Item	Ref	Experimental conditions	Gametes tested	Number of mutants	
				Observed	Expected
1	18	Control	688,921	39 (27.7–52.2)	40
2	4,9,18	300 R Acute X rays (used to compute β)	103,755	64 (48.7–80.3)	64
3	4,18	600 R Acute X rays (α and β)	119,326	111 (90.4–132.0)	239.1
a	12,13,18	670 R Acute X rays (α and β)	11,138	12 (6.7–20.3)	27.2
b	13,18	636 R Acute ^{60}Co γ rays (α and β)	12,021	11 (5.3–19.1)	26.8
c	15,18	600 R Acute ^{60}Co γ rays (α and β)	44,352	33 (22.9–45.3)	88.8
4	5,18	600 R Acute X rays (100+500, α and β 1-d interval)	24,811	42 (30.0–55.5)	38.6
5	13,18	626 R Acute ^{60}Co γ rays (α only, 60 daily doses 10 R)	23,982	7 (3.3–13.8)	8.6
6	13,18	600 R Acute X rays (12 \times 50 R, α and β , 1-week intervals)	18,119	16 (9.6–25.4)	8.8
7a	5,18	1,000 R Acute X rays (α and β)	44,649	29 (19.1–40.9)	229.5
b	4,18	1,000 R Acute X rays, 2 \times 500 R (α and β , 2-h interval)	14,879	12 (6.7–20.3)	76.5
8a	5,16,18	1,000 R (2 \times 500 R, α and β , 1-d interval) Acute X rays	16,626	55 (40.9–71.1)	47.2
b	17	1,000 R (2 \times 500 R, α and β , 4-d interval) Acute X rays	7,168	11 (5.3–19.1)	20.4
c	17	1,000 R (2 \times 500 R, α and β , 7-d interval) Acute X rays	8,271	11 (5.3–19.1)	23.5
d	4,15,18	1,000 R (600+400, α and β , 15-week interval) Acute X rays	4,904	10 (5.3–17.6)	14.4
9a	4,18	1,000 R (5 \times 200, α and β , 1-d intervals) Acute X rays	8,588	16 (9.6–25.4)	12.5
b	4,18	1,000 R (5 \times 200, α and β , 1-week intervals) Acute X rays	10,968	15 (8.1–23.8)	16.0

Numbers in parentheses are the 95% confidence limits for the observed number of mutations⁴⁴

lower than for the same dose (200 R) in item 2, but it has been suggested¹⁴ that the Harwell strain has possibly diverged from the Oak Ridge strain during their years of separation. (We think it most unlikely that the difference could be attributed to a dose rate effect¹⁴, since in item 2 the time for irradiation was approximately 2 min and in item 9, approximately 4 min).

Data from irradiated spermatogonia

For spermatogonia (Table 3), there was not a sufficient number of points at low doses to establish the shape of the dose curve. Therefore, to obtain the coefficients of mutation induction we adopted a different procedure than was used for irradiated females. The point at the lowest dose (300 R) was emphasised because differential cell killing and selection^{3,23} grossly distort the shape of the curve at high doses, for example, after 1,000 R fewer mutations are obtained than after 600 R. For the male, the linear regression coefficient, α , was obtained from a weighted linear regression analysis of all the extensive low dose rate data presented in Lyon *et al.*³⁵ and the control data¹⁸. The control data are given as item 1 in Table 2; the data summarised by Lyon *et al.* are not shown (this estimate of α , the control data, and the data obtained at 300 R (item 2) were used to calculate the value of β). The expected numbers were obtained from the equation as they were for the female data. All irradiation at less than 1 R min⁻¹ was assumed to be chronic and only the linear term was used to obtain expected values. The agreement is generally good for acute irradiation and for fractionated doses where individual doses are less than 500 R. The gross discrepancies at high acute doses, as noted before, are attributable to differential cell killing and selection. It is particularly noteworthy that induced rearrangements reflect a similar pattern in this dose range^{17,18}.

The agreement is reasonably good except in two circumstances (1) when the nominal dose instead of the effective dose is used to calculate the effect in females that were irradiated over long periods that extended into the early genetically insensitive stages, and (2) when high doses of acute irradiation, which cause differential cell killing or cell selection, were given to males.

This analysis leads to the suggestion that the similar kinetics observed for the induction of specific locus mutations and gross chromosomal aberrations is not fortuitous, but a reflection of both their common mode of origin and their subsequent common

response to modifying physiological factors. For instance, the analysis indicates that independent ionisations only interact to give a dose square term within a few hours, as has previously been found for chromosome breaks in other systems^{36,37}. Such breaks have been found to interact with one another over distances of the order of 1 μm . Reported values are 1.8–2 μm ⁴⁰, and 0.2 μm ⁴¹. An analysis of the size of the site within which the two events must occur to give a specific locus mutation, can be carried out by utilising the method of Kellerer and Rossi³⁹. This relates the target size to the dose where the linear and quadratic components are equal, that is, where αD equals βD^2 . This dose is given by c/β . In Table 2 we see the value of α/β is 104 in the male and is 111, 131, or 274 in the female depending upon which value for the control was used in the calculations. A corresponding value for the diameter of the site can be obtained directly from Fig. 5 of Kellerer and Rossi³⁹. The target radius is thus found to be approximately 0.55 μm , which is very close to the value obtained for chromosome aberrations⁴². This strengthens our belief that the specific locus mutations are indeed deletions. Moreover, Valcovic and Malling (personal communication) have been unable to induce point mutations that would be recognised as electrophoretic variants at nine protein-determining loci in mouse spermatogonia, although they have recovered a number of "null" mutants which, pending further analysis, they consider to be deletions.

The present method of analysis has heuristic value and highlights some mouse experiments that could be performed to distinguish this model from that invoking the repair of pre-mutational damage (and a dose or dose-rate dependent repair system that affects the shape of the curve). For instance, in the female, chronic irradiations lasting only 4–5 d at doses similar to those employed in the acute experiments, with matings restricted to one litter, should eliminate the problem of contamination by cells that were in the insensitive stage for a considerable portion of their radiation history. A simultaneous large scale control experiment in the female would help establish a more accurate spontaneous rate. Because it becomes critical to delimit the mutation rates for the different stages of oocytes, one other area of investigation that becomes highlighted by the present analysis is that of timed conceptions after female irradiations. In the male, experiments could be performed that would encompass acute

irradiation in the range of 100–500 rad. It is over this dose range that there would be a lesser tendency for extraneous factors to obscure the shape of the dose curve. This dose range, in addition, would provide information that would be useful for interpreting the saturation and differential cell killing phenomena encountered at higher doses (600 R to 1,000 R). We believe that the 300 R dose point listed above should be included because it is the point in common with previous experiments and could be used to ensure reproducibility of results.

Estimates of the mutation rate in mice have been used to determine the genetic hazards of radiation to humans^{19,20}. We believe that the values of α represent the mutation rate per rad to be expected under conditions of chronic irradiation where the two-hit component becomes negligible. For males this is the same value as obtained by a linear fit to the chronic data, the usual basis for human risk estimation.

Because the mutation rate obtained in mouse females drops to near zero (3/259,683) after the first three post-irradiation litters, when only eggs that had been irradiated as immature oocytes are sampled, it is often assumed²⁰ that in humans, too, chronic irradiation of the female over the whole pre-reproductive lifetime mainly will reach less mutable stages. Therefore the radiation-induced rate for females has been taken as essentially zero, and the overall risk from irradiation of both sexes has been taken as half the male mouse rate²⁰. It is unknown, however, whether or not the mutational response of the human oocyte is the same as that of the mouse. There is evidence for one effect of radiation, cell killing, showing that humans and other mammals differ from the mouse^{19,30}. In view of this, we, in agreement with the view expressed by Searle and Phillips⁴³, believe that the prudent, or conservative approach to take in estimating genetic risks is to assume that the mutations do not drop to zero in human females. This will tend to make the estimate of the risk somewhat higher, which, in view of the uncertainties involved, we believe to be the more responsible way to establish radiation safety standards.

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Spontaneous mutation by mutagenic repair of spontaneous lesions in DNA

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Strains of yeast carrying mutations in many of the steps in pathways repairing radiation-induced damage to DNA have enhanced spontaneous mutation rates. Most strains isolated because they have enhanced spontaneous mutation carry mutations in DNA repair systems. This suggests that much spontaneous mutation arises by mutagenic repair of spontaneous lesions.

WORK from the laboratories of Bessman¹ and Nossal², following the work, for example, of Speyer³ and Drake and Allen⁴, has established the idea that much spontaneous mutation in *Escherichia coli* and its phages arises by mistakes made by a polymerase/exonuclease complex during DNA replication. The 3'-exonuclease seems to have an editing function, scanning newly synthesised DNA for errors made by the polymerase, and variation in the amount of editing by the exonuclease is related to the frequency of spontaneous mutation.

In an analysis of mutants selected for mutator activity in

the yeast *Saccharomyces cerevisiae*, we have become aware of the inadequacy of the editing hypothesis, for these mutations occur at too many loci. Eight loci are definitely established, there is partial evidence for four more, and the system is clearly not saturated.

Induction of spontaneous mutations by natural agencies has been discussed ever since Muller⁵ and Stadler⁶ showed that X rays cause mutation. Intracellular production of mutagens⁷ to explain mutators⁸ and spontaneous occurrence of tautomeric changes in bases of DNA⁹ have also been suggested.

After the discovery that yeast had a higher spontaneous mutation rate in meiosis than in mitosis¹⁰, the possibility that errors were implicated in the other DNA metabolic processes, such as recombination¹¹ and replication^{12,13}, became widely accepted.

Ultraviolet and X-ray mutagenesis in *E. coli* require an intact repair system^{14–17}. The same applies to yeast for ultraviolet light^{18,19}, implicating the *RAD6* pathway, and for several chemical mutagens^{20,21}, again implicating the *RAD6* pathway, as well as *rad15*, which does not affect ultraviolet mutagenesis²².

Table 1 Mutator activity of *rad6-1* and *rad51-1*

(a) Tetrads segregating for <i>rad6-1</i>				
Tetrad no.	Reversion of <i>lys1-1</i> in UV-sensitive segregants		Reversion of <i>lys1-1</i> in UV-resistant segregants	
1	92	70	46	32
2	72	110	47	46
3	116	113	39	32
4	88	100	59	48
5	133	128	47	29
6	90		48	31
7	140	116	32	47
8	124	154	24	28
9	93		28	47
10	112	131	27	35
21	76	113	23	43
22	95	124	46	30
23	123	115	24	29
24	108	131	30	25
25	107	113	19	34
Mean of 28 <i>rad6-1</i> strains with standard deviation 110.24 ± 20.45			Mean of 30 <i>RAD6</i> strains with standard deviation 35.83 ± 10.14	
(b) Random meiotic products from tetrads segregating for <i>rad51-1</i>				
	Reversion of <i>lys1-1</i> in UV-sensitive segregants		Reversion of <i>lys1-1</i> in UV-resistant segregants	
	148		88	
	151		29	
	122		46	
	154		20	
	156		16	
			41	
			33	
			36	
			19	
			31	
			35	
Mean of 5 <i>rad51-1</i> strains with s.d. 146.20 ± 13.86			Mean of 11 <i>RAD51</i> strains with s.d. 35.82 ± 19.64	

The values given are the numbers of lysine-independent colonies arising during growth on plates with limiting lysine content²⁸. Each value is the average of two or more determinations, corrected for previously existing revertants. The method has been standardised with the fluctuation test method of determining reversion rates²⁹.

Study of the accumulated data on radiation sensitive and mutator mutants in yeast in the context that induced mutation is a repair phenomenon, reveals that spontaneous lesions susceptible to mutagenic repair are occurring in yeast.

Working model of repair processes in yeast

Radiations and many chemicals interact with DNA to produce a molecule which is locally not DNA. This chemically changed part of the DNA is known as a primary lesion. The primary lesion is a substrate for several systems of repair enzymes which recognise the distortion of the molecule and restore chemically sound DNA. In the case of ultraviolet-induced pyrimidine dimers it has been shown that one unrepaired lesion is a lethal event^{23,24}. We assume that a chemical alteration of DNA interferes with replication and/or function of the genetic material, and that most types of lesion are lethal.

The problem for repair systems is to know how to restore the informational content of the DNA. Photoreactivation of dimers is a chemical reversal of the lesion, so that information is never lost. Excision repair removes a length of the nucleotide chain containing the lesion and uses the complementary chain as a template for resynthesis^{25,26}. In some situations this solution is not available, perhaps because that enzyme system is already used to capacity, because of the configuration of close lesions, or because of the type of lesion involved. There is evidence for bacteria that replication past the site of a dimer makes it no longer amenable to excision repair²⁷.

In the cases not amenable to excision repair, the information may be taken from a sister chromatid or homologue. The effects of this may be seen genetically as induced crossing over or gene conversion. A further type of repair may use no record of the damaged information, or use one which cannot be copied accurately, so that the chemically sound DNA which is restored differs in its informational content from that which was originally damaged. The expression "error-prone repair"²⁸ is widely used for this kind of repair, but we prefer to call it "mutagenic repair" to avoid the implication that wrong information was inserted by mistake rather than as a necessity.

It is repair that causes the broad shoulder on the ultraviolet survival curve of wild-type yeast²⁷. At low doses there is very little cell death because all lesions are repaired by one or another repair pathway. At higher doses the repair capacities become saturated or inactivated, or are no longer capable of being induced^{28,29}, so that the killing curve becomes more nearly exponential as the kill becomes proportional to the induction of primary lesions.

Channelling of lesions

When a repair pathway is blocked, the lesions which would have been repaired by that pathway may be handled by the other repair capacities^{31,32}. If the pathway which is blocked is not a mutagenic repair pathway, it is to be expected that the strain will show a higher frequency of induced mutation per exposed cell at a given dose, because a higher proportion of the lesions is channelled into mutagenic repair.

It may be expected that all blocks affecting a first step in a pathway can channel lesions into other pathways, for the lesions remain unrepaired. If, however, the block is at an intermediate step in a repair pathway, the partially repaired lesion may not provide a suitable substrate for other repair capacities. This irreparable secondary lesion will then be lethal. In this situation, the kill will vary as the number of primary lesions accepted by the deficient pathway. Radiation-sensitive strains of this type will not be able to channel lesions into mutagenic repair. (They may, however, show an enhanced induced mutation frequency per survivor because the survivors were those cells which did not use the blocked pathway.)

It has been shown for yeast^{23,34}, and several other organisms³⁵, that some radiation-sensitive strains are mutators, while others are not. If radiation-sensitive strains derive their mutator activity from channelling spontaneous lesions into mutagenic repair pathways, it is to be expected that first steps in pathways would all be able to channel lesions, and therefore be mutators.

There are three first steps of repair pathways for ultraviolet-induced lesions in yeast³⁶, *rad3*, *rad51* and *rad6*. Brychey found³⁷ that *rad3* was a mutator, while later steps in the excision repair pathway³⁸ (*rad1*, *rad2* and *rad4*) were not. Table 1 shows that *rad51* and *rad6* are also mutators. Thus blocks in all three first steps cause enhanced

Table 2 Sensitivity of mutator mutants at eight loci to the lethal effect of three mutagenic agents

	X rays	UV	MMS*	No. of alleles tested
<i>mut1</i>	R	R	R	6
<i>mut2</i>	R	R	S	11
<i>mut3</i>	R	WS	S	2
<i>mut4</i>	R	WS	S	1
<i>mut5</i>	S	WS	S	1
<i>MUT6</i>	R	R	R	1
<i>mut9</i>	WS	WS	S	1
<i>mut10</i>	S	R	S	1

R, wild-type level of resistance; S, sensitive; WS, weakly sensitive; MMS, methyl methanesulphonate.

*These results were obtained by Anwar Nasim and Theresa Brychey.

spontaneous mutation, while blocks in later steps may or may not show mutator activity.

These observations lead to the conclusions that all three pathways are involved in the repair of spontaneous lesions, and that when any of these pathways is unable to "accept" a lesion there is an increased probability of the lesion being repaired by a mutagenic repair pathway.

It is especially interesting that *rad6* is a mutator because this is the first step of the pathway responsible for most ultraviolet and X-ray-induced mutations²⁷, and for most or all mutations induced by a variety of chemical mutagens²⁰. We interpret this to mean that another mutagenic pathway repairs spontaneous lesions and leads to mutation with a higher efficiency than the *RAD6* pathway. The alternative interpretation, that the *rad6* mutant is leaky, and mutated in such a way that it is more likely to make mistakes, is discounted because the *rad6-1* allele used is amber suppressible and therefore unlikely to have any function in the mutant protein. Lawrence *et al.*¹⁹ showed that *RAD6* is predominantly responsible for transitions, which implies that another pathway causes other types of mutation.

Sensitivity of mutator strains and recessiveness of mutations

Since repair pathways show little specificity in the types of lesion on which they act, it is to be expected that strains with blocks in pathways involved in the repair of spontaneous lesions will show sensitivity to lesions induced by mutagenic agents. If mutator mutations act by channelling spontaneous lesions into mutagenic repair pathways, it should be possible to confirm that the genes are involved in repair of damage to DNA by identifying the sensitivity of the mutants to mutagenic agents.

The results obtained so far are summarised in Table 2. Only *mut1* and *MUT6* fail to show sensitivity to one or more of the agents tested.

Loss of a repair capacity is expected to be recessive, and the radiation sensitivity of *rad* mutations has been found to be recessive⁴⁰. In contrast, it is to be expected that genetic control of the frequency of errors made during DNA synthesis will be incompletely dominant. This is clearly the case if the lesion is in the polymerase itself. If the mutator mutation affected an exonucleolytic edition function¹, the mutator activity would still be incompletely dominant if the 3'-exonuclease were a part of the polymerase. Recessiveness of such an altered exonuclease function could be found only if the wild-type exonuclease were free to scan all replication sites, and the mutant exonuclease were absent.

For these reasons we take the recessiveness of a mutator mutation to be strong support for the idea that it acts by modifying the repair capabilities of the cell, and not by changing other DNA metabolic processes.

Table 3 shows determination of dominance for the mutator mutations. Most mutators are less effective in a homozygous diploid than in a haploid. We attribute this to a mode of repair present in a/α diploids and absent from haploids. It can be said that all alleles tested at seven of the eight loci are recessive, and that *MUT6* shows dominance in some backgrounds.

Implications

The argument presented here can be summarised as follows. Radiation-sensitive mutants show induced mutation frequencies which differ from those found in the wild type because the mutations cause changes in the proportions of primary lesions which are channelled into various repair pathways. Some of the repair pathways are mutagenic and others are not.

That these same radiation-sensitive mutations affect the frequency of spontaneous mutation is taken as evidence that spontaneous lesions occur, and are repaired by systems having many steps in common with the systems repairing

induced lesions. It can also be concluded that mutagenic repair is available to the spontaneous lesions.

When strains of yeast are isolated which have increased spontaneous mutation rates, it is found that they represent mutations at many more loci than would be expected to be involved in DNA synthesis, that most of them are recessive and that most of them show sensitivity to mutagens. These points taken together lead to the conclusion that the mutators enhance spontaneous mutation rates by channelling spontaneous lesions into mutagenic repair pathways. The only candidate among these strains for a mutator acting on other DNA metabolic processes is *MUT6*, which is dominant and not known to be sensitive to any mutagenic agent. Another possible case was reported by Esposito *et al.*⁴¹, who found that *spo7*, which seems to be involved in premeiotic DNA synthesis, was an antimutator.

Table 3 Mutator activity in diploids homozygous and heterozygous for alleles at eight loci

Locus and allele	<i>mut</i> —	<i>mut</i> —	+
	<i>mut</i>	+	+
<i>mut1-1</i>	657	43	31
-2	761	131*	61
-4	455	34	27
-5	253	34	30
-6	417	50	30
<i>mut2-1</i>	158	55*	61
-2	274	41	61
-5	389	58	61
-6	188	28	27
-7	186	31	30
-8	139	26	30
-9	337	46	20
-10	185	23	20
-11	346	24	18
<i>mut3-1</i>	168	53	31
-2	125	55	20
<i>mut4-1</i>	96†	34	31
<i>mut5-1</i>	265	36	20
<i>MUT6-1</i>	182	51‡	18
<i>mut9-1</i>	280	32	20
<i>mut10-1</i>	84	18	19

Values given are as for Table 1. They are the average of those obtained from at least four independently isolated diploids, except in the cases noted below.

* Values determined from two independently isolated diploids.

† Value is the average of 38 independently isolated diploids. The values obtained ranged from 30 to 270, so that the mutator activity could not be detected in some homozygous *mut4-1* diploids.

‡ This value is the average of 24 independently isolated diploids. The values obtained ranged from 14 to 229, so that *MUT6-1* was seen to be dominant to the wild-type allele in some heterozygotes, and recessive in others.

An explanation of mutator activity in terms of channelling of spontaneous lesions leads to the conclusion that the frequency of spontaneous lesions is higher than the frequency of spontaneous mutations in wild-type strains, and is therefore high enough to account for all spontaneous mutation. We also conclude that mutagenic repair is not induced by the presence of mutagenic agents, but is constitutive, at least in some cells. Lemontt⁴² has published evidence that such repair is constitutive in *Ustilago*.

Whether any part of the spontaneous mutation frequency of wild-type cells can be attributed to mistakes in replication can be determined by isolating antimutator strains which have lost all mutagenic repair capacities. A further problem raised by these conclusions is the origin of the spontaneous lesions, which may be either endogenous or naturally induced. One possibility is that replication errors provide a substrate for repair, and are therefore a source of spontaneous lesions.

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Somatic mutation as the basis for malignant transformation of BHK cells by chemical carcinogens

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The chemical induction of malignant transformation in BHK cells seems to result from a somatic mutation. Stable transformants, whose frequency is significantly increased by mutagenic carcinogens, can revert to normal and often display temperature-restricted phenotypes indicative of an altered gene product.

ALTHOUGH the many alterations in cell growth, morphology and biochemistry which accompany the chemical induction of malignant transformation in cultured mammalian cells have been extensively described, the primary event by which the inducing carcinogen causes these changes is unknown^{1,2}.

Two general mechanisms have been proposed to explain how this initial cellular transformation occurs during chemical carcinogenesis. On the one hand transformation may be the result of an epigenetic change in the expression of a gene or activity of a gene product which is not due to an alteration in the primary structure of DNA. Such changes are presumed to be responsible for much of development, including the activation-inactivation of the mammalian X chromosome³ and the extinction and activation of differentiated functions which have been observed in cultured cells⁴. The ability of markers from embryonic tumour cells to reappear in normal differentiated adult tissues⁵ lends support to this idea that transformation results from a change in gene function rather than in gene structure.

Alternatively, transformation may be the result of a somatic mutation, defined here in Siminovitch's global sense⁶ as a heritable change in nucleotide sequence resulting from an alteration, deletion or rearrangement in the primary structure of cellular DNA. Most of the chemicals known to cause cancers in animals are mutagenic, their active forms interact with DNA *in vivo* and *in vitro*^{7,8} and cause mutation in bacteria (refs 9 and 10 and D. Anderson

et al., quoted in ref. 8) and occasionally in mammalian cells^{11,12}. It is not clear, however, whether the observed interactions of carcinogens with DNA reflect the mutagenic nature of the events underlying their carcinogenicity or merely their almost universal electrophilic nature¹³. To show that mutagenicity is the mechanism by which chemicals induce malignancy, it is necessary to go beyond the demonstration that carcinogens are capable of being mutagens and to show that transformation itself is due to a carcinogen-induced mutation.

In this paper we attempt to do this by investigating malignant transformation *in vitro*, using the established quasi-diploid hamster line BHK21/cl 13 and the chemical carcinogens nitrosomethylurea and 4-nitroquinoline-1-oxide. Cloned cell cultures are exposed briefly to a carcinogen, grown for 3 d in liquid culture and then assayed by plating in soft agar. Cells able to form colonies in the soft agar are considered to be malignant transformed since this characteristic of anchorage independence *in vitro* shows a complete correlation with *in vivo* malignant growth^{14,15}. Whether or not these newly induced transformed cells are the result of mutations cannot be determined directly because the usual techniques of genetic recombination, DNA physical chemistry and sequence analysis of an altered gene product cannot yet be usefully applied to cultured mammalian cells. A number of other testable criteria, however, have come to be accepted^{6,16,17} by which a newly occurring phenotype in a somatic cell can be defined as a mutation. These criteria are (1) the phenotype should arise with a low frequency spontaneously; (2) this frequency should be increased by mutagenesis; (3) the phenotype should be associated with an altered gene product, usually a protein; (4) the phenotype should be stable; (5) reversion should be low but demonstrable; (6) the phenotype should be localised on a chromosome. The data presented below demonstrate that the induction of the transformed phenotype by carcinogens in cultured BHK cells fulfils the five of these six

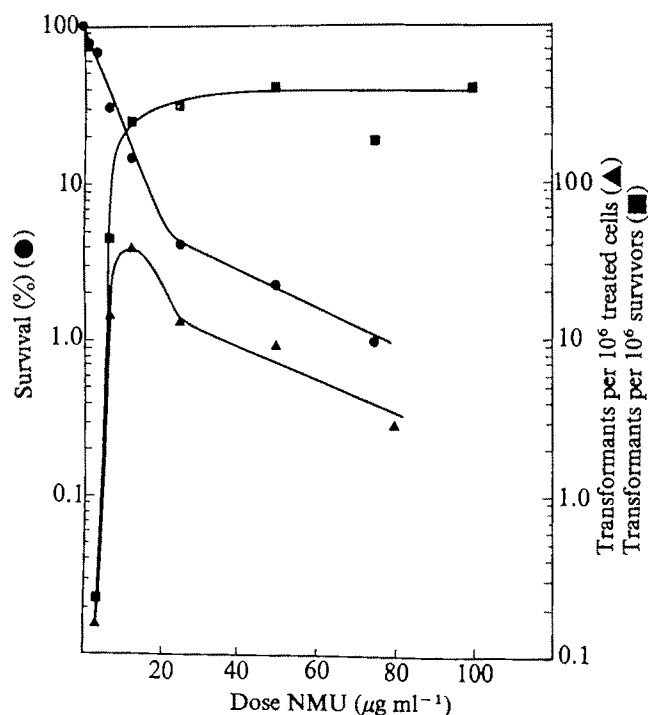


Fig. 1 Inactivation and transformation by nitrosomethylurea. BHK Supernormal cl 10 cells were washed and treated for 1 h at 37 °C with various doses of freshly prepared NMU (synthesised for these experiments) while suspended at 2×10^6 cells per ml in TD buffer¹⁸ containing 10% of the 5 mM citrate buffer (pH = 5.5) which served as carcinogen solvent. Following treatment cells were washed and assayed for survival in high serum growth media (Dulbecco's MEM + 10% tryptone phosphate broth + 20% calf serum) by measuring colony forming ability compared to that of cells treated with solvent only. There was no decrease in survival after treatment with solvent alone. Transformation was assayed following 3 d sub-confluent growth in liquid culture, during which control cells underwent six doublings, by plating in growth medium partially solidified with 0.34% agar at a density of 1×10^6 cells per 60-mm dish following the technique of Macpherson and Montagnier¹⁹. Transformants were counted as colonies growing to a diameter ≥ 0.2 mm after 2-3 weeks incubation at 38.5 °C. Concurrent assay of liquid colony formation allows one to measure the frequency of transformants per survivor at the end of the expression period. The number of transformants per treated cell was calculated by multiplying transformants per survivor times the survivors per treated cell measured immediately following carcinogen treatment.

requirements tested so far and thus seems operationally to be a mutagenic event.

Low spontaneous frequency of transformation

Normal laboratory stocks of the established line of baby hamster kidney cells, BHK21/cl 13 (ref. 18), frequently show a high background of colonies, $\leq 5 \times 10^{-4}$, when plated in soft agar. This is apparently due in part to the accumulation of spontaneously transformed variants and their enrichment when the stocks reach confluence at each passage¹⁹, and in part to a propensity for spurious colony formation at high cell densities. To avoid both these complications this line was cloned in liquid and a subline selected, BHK supernormal clone 10 (BHK SN cl 10) which clones in liquid with a normal efficiency of 50-60% but fails to form any colonies in soft agar at densities of up to 2×10^6 cells per 60 mm dish. This subline has not been tested yet for *in vivo* tumorigenicity. The frequency of spontaneous transformation (below) suggests that it would be tumorigenic at sufficiently high doses, as is its parent line¹⁸. Aliquots of this subclone were used for most experiments reported here. To minimise selection for spontaneously transformed variants, cells were not allowed to become confluent during growth.

The frequency of spontaneously arising transformants was determined at the seventh passage after recloning by assaying 10^4 untreated cells in soft agar; techniques are described in the legend to Fig. 1. Colonies arising in agar were picked, grown in liquid culture and retested in agar before being counted as transformed. No unstable transformants were observed. The frequency of spontaneously arising stable transformants was 1.3×10^{-7} per tested cell and 2.2×10^{-7} per cell able to clone in liquid. When this cloned cell line was tested earlier at fifth and sixth passage levels as controls in carcinogen experiments, no spontaneous transformants were observed (less than one transformant per 10^7 cells tested per experiment).

These frequencies of transformation underestimate the true number of transformants arising in these experiments because transformed cells do not clone with 100% efficiency in agar. The average relative plating efficiency in agar (% of cells cloning in liquid which are able to clone in agar) of the spontaneously transformed clones is 25%. When corrected for this, the spontaneous transformation frequency is 8.8×10^{-7} per viable cell. Both the observed and corrected frequencies of transformants are well within the range of frequencies observed for spontaneous mutations at well defined loci in other mammalian cells ($\sim 10^{-8}$ to $< 0.5 \times 10^{-7}$)^{17,20,21} and thus compatible with their origin as random mutations.

Carcinogens increase transformation frequency

When aliquots of the BHK SN cl 10 line are treated with either of two mutagenic carcinogens, the frequency of transformed cells is dramatically increased. Figure 1 illustrates the inactivation and transformation induced by nitrosomethylurea (NMU), a powerful carcinogen²² which spontaneously produces DNA alkylating intermediates²³ and is a base-changing mutagen in bacteria^{9,10}.

The survival curve for NMU is consistently biphasic,

Fig. 2 Inactivation and transformation by 4-nitroquinoline-1-oxide. BHK Supernormal cl 10 cells were washed, treated and assayed as described in Fig. 1 except that the carcinogen was NQO (ICN Pharmaceuticals, Plainview, New York); its solvent, DMSO, was present during treatment at a concentration of 1%; and cell concentration during exposure to carcinogen was 1×10^6 ml⁻¹.

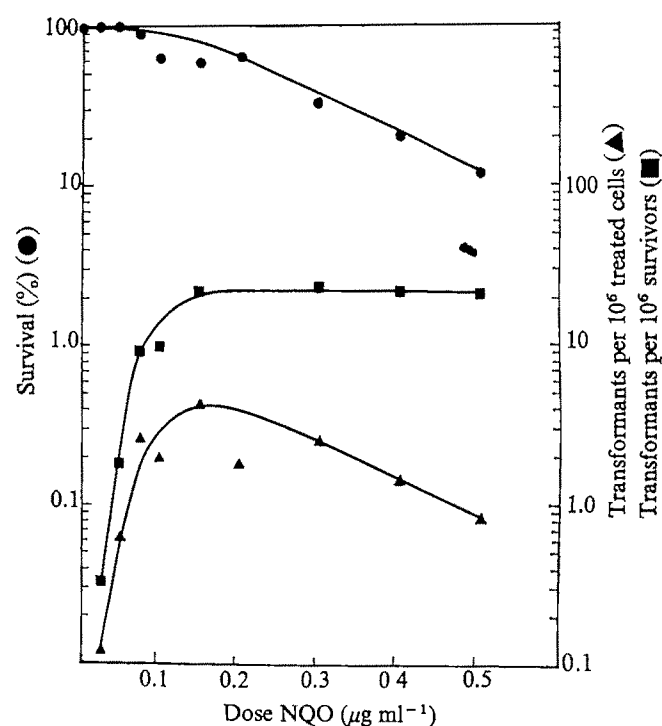


Table 1 Expression of the normal phenotype by clones of BHK SN cl 10 transformed by different doses of nitrosomethylurea

Dose NMU ($\mu\text{g ml}^{-1}$)	Number of clones in which normal phenotype is		
	Cold-sensitive	Heat-sensitive	Unexpressed
6.25	1	3	2
12.5	0	4	3
25	3	0	3
50	0	2	9
75	1	4	3
Total	5	13	20
% of total tested clones	13%	34%	53%

Transformants were isolated as individual clones arising in agar following the carcinogen treatment outlined in Fig. 1. Several colonies from each dose were grown in liquid and recloned in either liquid or soft agar at 38.5 °C. Following this second cloning, cells were split, grown in log phase at either 32 °C or at 38.5 °C for a minimum of 7 d to eliminate phenotypic lag¹⁸ and assayed at the same temperature for ability to clone in high serum liquid growth media and in growth media solidified with 0.3% agar¹⁹. 800 total cells were tested in liquid, 10⁴ cells in agar. A clone was considered to be heat sensitive for the expression of its normal phenotype if its absolute plating efficiency in soft agar (agar colonies per cell plated) at 38.5 °C was more than 10 times greater than that at 32 °C and if its relative soft agar plating efficiency (agar colonies per cell able to clone in liquid) was more than seven times greater at 38.5 °C than at 32 °C. A clone was considered to be cold sensitive for expression of the normal phenotype if the converse of these criteria was met. The clone was considered transformed and the normal phenotype unexpressed at either temperature if the absolute agar plating efficiencies were more than tenfold greater than the normal controls and differed by less than tenfold at the two temperatures and the relative agar plating efficiencies differed by less than sevenfold.

perhaps reflecting killing by the cyanate ion breakdown product of NMU²⁴ as well as by macromolecular alkylation. NMU transforms efficiently; the maximum transformation frequency observed is 3.8×10^{-6} transformants per treated cell, 4×10^{-4} transformants per survivor, an almost 300-fold increase over the spontaneous frequency. Correcting for the 15% average relative agar plating efficiency of isolated and purified NMU-induced transformants, the frequency per survivor becomes 2.2×10^{-5} .

Killing and transformation induced by 4-nitroquinoline-1-oxide (NQO) is illustrated in Fig. 2. NQO is a free radical carcinogen²⁵ which is activated by cellular enzymes to intermediates which form NQO-purine adducts in DNA^{26,27}. It is an effective mutagen in bacteria where it causes both base changes and reading frame shifts^{9,10,28} and in yeast where it shows a specificity for GC pairs⁹. NQO lesions undergo extensive repair in bacteria^{26,27} and mammalian cells²⁹, which is reflected in the shoulder of the survival curve.

NQO transforms effectively, even at doses where survival is unaffected. The maximum transformation frequency observed is 4×10^{-6} per treated cell, 2.2×10^{-4} per survivor, a 30-fold increase over the spontaneous frequency. If corrected for the 25% average relative agar plating efficiency of isolated NQO-transformed clones, the frequency per survivor becomes 8.8×10^{-5} .

This frequency is lower by two orders of magnitude than that observed by Kakunaga when he used similar doses of NQO to transform a BALB/3T3 mouse cell line^{31,32}. His higher frequencies may be due in part to the use of transformed foci as an assay for transformation. Although he finds that five of the focus-derived NQO-transformed lines clone in soft agar and are tumorigenic³², we have found only one in 10 morphologically transformed colonies to be truly transformed by these criteria¹⁵, and others also find that morphological conversion can occur without tumorigenicity¹⁴.

Transformation observed in response to these two carcinogens does not appear to result from selection of pre-existing transformants which are more resistant to the inhibiting or lethal effects of the carcinogens, since (1) cells are treated with the carcinogens only briefly while sus-

pended in buffer under non-growing conditions so no selective overgrowth is possible while the carcinogen is present; (2) transformation is observed at a non-toxic concentration of NQO; and (3) the frequency of transformants per treated cell falls in parallel with survival indicating that transformed variants are as sensitive to inactivation by the carcinogens as the normal cells. In the case of NMU, this parallel is obscured at doses less than $15 \mu\text{g ml}^{-1}$ by the rapidly increasing number of induced transformants.

An expression time of about six cell generations is allowed between carcinogen treatment and assaying for induced transformants, to give time for fixation of any possible pairing errors and for phenotypic expression of transformation. Temperature shift experiments with BHK cell lines temperature sensitive for normal growth have shown that this expression requires 3.5 generations¹⁸. It is doubtful that the cell population becomes enriched for transformants during this time, as transformed lines grow with the same doubling time as normal BHK cells¹⁵, and confluent conditions where they might be at an advantage are scrupulously avoided.

At lower doses where transformation frequency per survivor rises with increasing carcinogen dose, the dose-response relationship is linear. A levelling off of the frequency of transformants occurs at higher doses, as has been observed previously for NMU²⁴ and for other carcinogens²². The maximum frequencies of transformation observed are within the range of frequencies with which mutations are induced by mutagens at other, better characterised loci in mammalian cells^{30,31,34}. For example, more than 9.7×10^{-4} 8-azaguanine-resistant mutants per survivor are induced by NMU in Chinese hamster line²².

Induction of temperature-dependent transformants

This laboratory has previously reported the isolation of several chemically transformed derivatives of BHK which were selected for growth in soft agar and shown to be malignant *in vivo* yet *in vitro* behaved as normal cells at 32 °C and as transformed only at 38.5 °C (ref. 15). They were considered to have a heat-sensitive lesion in some gene product necessary for normal growth rather than a cold sensitive lesion in a product causing transformation, because they occur at low frequency and in the first case the change from the normal phenotype can be accomplished in one step, whereas in the second case two steps are required—the turning on of whatever is required for expression of transformation plus a second event to make transformation cold sensitive. This convention will be followed here.

In the current series of experiments transformed colonies resulting from the carcinogen treatments depicted in Figs 1 and 2 were selected, purified by recloning, and split to

Table 2 Expression of normal phenotype by clones of BHK SN cl 10 transformed by different doses of 4-nitroquinoline-1-oxide

Dose NQO ($\mu\text{g ml}^{-1}$)	Number of clones in which normal phenotype is		
	Cold-sensitive	Heat-sensitive	Unexpressed
0.05	0	3	1
0.075	0	3	4
0.10	0	1	6
0.15	0	6	2
0.20	0	4	4
0.30	0	1	6
0.40	0	4	1
0.50	0	2	5
Total	0	24	29
% Total tested clones	0%	45%	55%

Transformants were isolated following carcinogen treatment as outlined in Fig. 2 and grown and tested for the expression of their normal phenotype as described in Table 1 except that all reclonings were done in soft agar (0.34%).

high and lower temperatures where they were grown and tested for the expression of the normal phenotype assessed as a significant depression in absolute and relative plating efficiency in soft agar (Tables 1 and 2). At almost every dose some clones remain fully transformed at both temperatures. Fifty-three per cent of the NMU-transformed clones and 55% of the NQO-transformed clones display this unconditional phenotype. The remainder of clones tested express the normal phenotype at one of the temperatures and the transformed phenotype at the other. The majority of these temperature restricted clones are transformed at the high temperature, normal at the low, as is expected since transformants were initially selected as clones growing in agar at 38.5 °C. However, at three different doses of NMU clones arise which are normal at 38.5 °C and transformed at 32 °C. No such clones cold sensitive for the normal phenotype were seen following NQO treatment. This is probably because the second cloning was performed in agar at 38.5 °C, rather than in liquid or agar as with NMU; this imposes a second selection against such variants which do not plate efficiently in agar at high temperature.

Fourteen spontaneously arising transformants were similarly tested and none were found to be temperature dependent for transformation. However, as they all come from aliquots of the same culture, they may be sibs and represent only one transformation event. Another spontaneous BHK transformant isolated earlier in this laboratory is normal at 32 °C and transformed at 38.5 °C (ref. 15).

Due to the period of growth between the removal of the carcinogen and the assay for transformation, many of the transformed clones isolated within a single dose in these experiments may also be derived from a single transformation event. However, the minimum number of clones of independent origin can be tabulated as shown in Table 3 using data from several experiments and three carcinogens. Even with this correction, 57% of the clones are temperature restricted.

The high frequency of temperature-limited phenotypes is surprising but not unique. In *Escherichia coli* 75 out of 150 independent thymine-less mutants isolated at 37 °C were temperature sensitive, requiring thymine for growth at 37 °C but not at 28 °C³⁸. In *Saccharomyces* 12 out of 26 spontaneous *ade-3* mutants were temperature sensitive³⁹. In *Drosophila*, one half of a group of newly isolated EMS-induced notch mutants were temperature sensitive and one-quarter of existing mutants at this locus are temperature sensitive³⁷. In mammalian Chinese hamster cells 25% of cells selected for spontaneous 6-thioguanine resistance at high temperature could grow in HAT medium at low temperature, suggesting a temperature-sensitive HPRT enzyme defect which was verified *in vitro* for three clones³⁸. Certain loci in a variety of organisms seem to have particular sensitivity for temperature-sensitive lesions; the locus or loci involved in the transformed phenotype in BHK may be one of these types.

An unusually high frequency of temperature-limited phenotypes is also often found when screening for revertants of bacterial³⁹ and mammalian^{40,41} cell mutants. If the induction of transformation is the result of an alteration in a gene product whose function is vital to the cell, then perhaps the number of sites where transforming lesions can occur is limited, as is the number of sites where a change can restore function to produce a revertant. The presence of a temperature-sensitive site among these few permissible lesions could explain the high numbers of temperature-restricted phenotypes among transformants and revertants.

Temperature- and cold-sensitive transformed clones may not be unique to the BHK 21 cell line. Clones similar in some respects to those reported here have been isolated directly from BALB/c-3T3⁴² and as mutagen-induced revertants of transformed lines of rat liver⁴³ and Chinese hamster lung⁴⁴.

In most cases carefully analysed so far, temperature-restricted phenotypes have been found to be the result of a temperature-sensitive or cold-sensitive gene product, typically a protein derived from a missense mutation⁴⁵. Thus these carcinogen-induced temperature-limited transformants are most plausibly explained as resulting from carcinogen-induced missense mutations in a gene specifying some product necessary for normal cell growth. It is particularly difficult to explain by epigenetic mechanisms the induction in the same cells, in the same tube at the same time by the same carcinogen, of three distinct transformed phenotypes as is the case for two doses of NMU (6.25 and 75 µg ml⁻¹, Table 1), whereas these situations are easily explained by mutations at three different sites in a gene or genes regulating normal growth.

Stability of transformants

The transformants described above are stable both *in vitro* and *in vivo*. Temperature-restricted clones isolated several years ago¹⁵ have been grown extensively, recloned in liquid and in agar and always found to retain their original characteristic temperature sensitivity for expression of the normal phenotype. One of these clones, Me₂N4 (induced by dimethylnitrosamine), plated in soft agar with an efficiency of less than 0.01% at 32 °C and 6.1% at 38.5 °C when first isolated¹⁵. In a recent re-test, a minimum of 60 generations and four years later, it plated in soft agar at 0.02% at 32 °C and 27% at 38.5 °C. A clone isolated during the current experiments, NMU no. 19, plated in soft agar with an efficiency of less than 0.03% at 32 °C and 15% at 38.5 °C at the second passage after isolation. Eight passages (~56 cell generations) later, it plated in soft agar at less than 0.03% at 32 °C and 18% at 38.5 °C. *In vivo* stability was demonstrated by Dr Arthur Soller in this laboratory with cells recovered from a tumour which had been induced in Syrian hamsters by subcutaneous injection of Me₂N4. When the tumour cells were returned to culture, they were found to retain their original phenotype—transformed at 38.5 °C, but normal and unable to clone in soft agar at 32 °C.

Reversion to normal phenotype

The frequency with which the cell line Me₂N4 (ref. 15) (which is temperature sensitive for the expression of the normal phenotype) reverts to normal following ethylmethane sulphonate (EMS) mutagenesis was determined by growing EMS-treated cells at 38.5 °C in methylcellulose containing

Table 3 Clones of independent origin

Carcinogen	Expression of normal phenotype			Total clones
	Heat-sensitive	Cold-sensitive	Un-expressed	
NMU no. clones	8	3	8	19
%	42	16	42	
DMN no. clones	2	0	0	2
%	100	—	—	
NQO no. clones	8	0	8	16
%	50	—	50	
none no. clones	1	0	1	2
%	50	—	50	
Total clones	19	3	17	39
%	49	8	43	100

Clones are considered to have originated independently if: (1) they occurred in response to different doses of carcinogen in an experiment where the similarly treated and tested control culture showed no detectable transformants or (2) they occurred at the same dose, but have significantly different phenotypes, that is, cold-sensitive, heat-sensitive or unrestricted DMN = dimethylnitrosamine. The isolation of DMN clones was reported previously¹⁵. Of the spontaneously arising transformants, one was isolated previously¹⁵, the other during the course of this work in a large experiment on BHK SN cl 10 performed as outlined in legend to Fig. 1 but omitting the carcinogen treatment. They were re-cloned and analysed as described in the legend to Table 1.

Table 4 Selections by lethal growth in methylcellulose-FUdR

	BHK		Me ₂ N4	
	No FUdR	+ FUdR	No FUdR	+ FUdR
Plated in methylcellulose				
Total cells	1.1×10^6	1.1×10^6	1.3×10^6	1.5×10^6
Total colony formers	1.7×10^4	1.7×10^4	5.7×10^4	1.1×10^5
Recovered from methylcellulose				
Total cells	7.5×10^5	6.2×10^5	8.7×10^5	5×10^5
Total colony formers	1.0×10^4	6.4×10^4	2.6×10^4	9.8×10^3
Corrected colony formers	1.3×10^5	1.1×10^5	3.7×10^5	3×10^4
% Colony formers surviving treatment	79%	68%	65%	2.7%
% Colony formers surviving second cycle	ND	ND	ND	15%
% Colony formers surviving third cycle	ND	ND	ND	12%

Me₂N4 is a chemically transformed cell line which is temperature-sensitive for the expression of the normal phenotype; BHK is its parent line, BHK21/cl 13. Selections in methylcellulose were carried out by plating 10 ml of well-dispersed cells suspended in growth media containing 1.2% methylcellulose (Fischer, 4,000 centipoise) atop a 15 ml base layer of growth medium solidified with 0.5% agar. One-hundred millimetre bacteriological plastic dishes were used to prevent cells from growing on the sides of the dishes. Plates were incubated for 3 d at 38.5 °C in a humidified atmosphere containing 10% CO₂. Where indicated, 5-fluorodeoxyuridine (FUdR, final concentration 125 µg ml⁻¹) and uridine (final concentration 500 µg ml⁻¹) were added at the end of day 1. Treatment was terminated at the end of d 3 by adding 10 ml growth media containing thymidine (Final concn 28.6 µg ml⁻¹). After 30 min further incubation, the slurry was removed, the agar base layers scraped and washed with an ice-cold mixture of 1:1 growth media and versene (0.02% disodium dihydroxy versenate in phosphate buffer). The cells were collected from the diluted slurry, washed by centrifugation, counted and assayed for colony formation in growth media containing 20% calf serum. Corrections were made for any loss in cell numbers during recovery from methylcellulose. In the case of the Me₂N4+FUdR, the cells were mutagenised by stirring for 1 h at 37 °C in TD buffer with 2.42×10^{-3} M ethylmethylsulphonate (Kodak), washed and grown at sub-confluent density for 2 d in liquid at 38.5 °C before methylcellulose selection. These cells were cycled three times through the methylcellulose-FUdR selection. They were grown in liquid for 2 d at 38.5 °C between each cycle. ND, Not determined.

5-fluorodeoxyuridine to enrich for normal revertants (Table 4). Over 90% of the transformed cells are killed in 2 d growth in FUdR-methylcellulose, whereas normal cells are not significantly affected by the FUdR and survive as well as transformed cells survive in methylcellulose in the absence of FUdR. The mutagenised cells were subjected to three cycles of methylcellulose-FUdR selection with 2-d non-selective growth periods in between. At the end, single colonies were randomly isolated from liquid culture, grown and tested for the expression of the normal phenotype at 38.5 °C shown as the ability to clone in liquid but not in soft agar. In several experiments a total of four stable revertants have been isolated. Knowing the number of viable colony formers at each selection step from plating efficiencies and assuming no differential loss of normal revertants during the experimental procedure, the number of revertants induced by EMS in the original population can be estimated to be 4×10^{-6} per treated cell, 1×10^{-8} per survivor. No revertants were observed without EMS treatment. This reversion frequency is within the range observed for the reversion by *N*'-nitro-*N*-nitrosoguanidine of temperature-sensitive mutations for growth in the BHK cell line ($0.5\text{--}5 \times 10^{-5}$ per cell tested)⁴⁶ and for the reversion by activated carcinogens of a well known temperature-sensitive leucyl-tRNA synthetase CHO cell mutant (2.1×10^{-5} to 4.9×10^{-4} temperature-resistant revertants per survivor)⁴⁷.

In summary, with respect to the characteristics analysed thus far, the chemically-induced BHK transformants are similar to many well documented somatic cell mutants. Although a chromosomal location has not yet been established, the most reasonable explanation for these transformants is that they result from a somatic mutation. The alternative hypothesis that they are of epigenetic origin appears unlikely. In other cases where epigenetic mechanisms have been evoked to explain heritable variations in cultured somatic cells the data have been found unconvincing^{48,49}, and in one instance genetic mechanisms have been shown to be able to explain aberrant results sometimes attributed to epigenesis⁴⁹.

Experiments suggestive of epigenetic carcinogenesis, such as the demonstration that markers from malignant teratocarcinoma cells injected into blastocysts subsequently show

up in normal adult tissue⁵, can be explained in other ways—for instance, by differentiation of the totipotent transformed cells to a state insensitive to the original transforming mutation⁵⁰ or by marker rescue through *in vivo* cell fusion⁵¹. Our findings that (1) three different transformed phenotypes can result from a single treatment of one aliquot of recently cloned cells and (2) half of the total induced transformants are temperature limited in the expression of their transformed phenotype are hard to account for by epigenetic mechanisms, whereas they are easily explained by mutagenesis.

The nature of the gene product which is rendered heat- or cold-sensitive in a temperature restricted transformant is not known. The simplest hypothesis is that it is some product necessary for normal, non-malignant growth. It could act directly or through repression of some oncogene, but the low reversion rate makes involvement of an oncogene unlikely. (Work from this laboratory, to be published soon, indicates that the reversion rate is significantly higher in virus-transformed BHK lines where an oncogene is known to function.) The gene product could be involved in viral transformation although the finding that several temperature-limited clones can be transformed by both RNA and DNA tumour viruses with normal frequencies at the temperature where they display the normal phenotype⁵² suggests that this may not be the case.

A single base change missense mutation is the most likely mutagenic event responsible for the transformation of BHK cells. But the high frequencies with which transformation, an apparently recessive trait in rodent and other cells⁵³, is detected in this nearly diploid line suggest that the line is or becomes functionally haploid at the locus or loci involved in transformation. If a transforming mutation does require a somatic crossover or chromosomal rearrangement or segregation for expression, then these events must be remarkably frequent to account for the high frequency of transformation. The possibility that the carcinogen is not inducing a point mutation at all, but rather is increasing the frequency of chromosomal aberrations which permit the expression of pre-existing alleles is very unlikely. Despite an extensive search, no consistent chromosomal changes have been associated with chemically induced transformation in other Syrian hamster cells^{54,55}. Our observations of three

distinct phenotypes coupled with the wide range of agar plating efficiencies which occur within each phenotype argue against this theory in that the number of pre-existing alleles capable of causing transformation would be excessively large.

Thus, although chemical carcinogenesis in these and other mammalian cells is far from being clearly understood, the evidence which is now available in the case of BHK cells is consistent with the hypothesis that a somatic mutation is the primary event by which chemicals transform normal cells into ones capable of malignant growth.

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letters to nature

Direct observation of individual cosmic ray showers

THE detection of the cores and directions of large cosmic ray showers became possible in the late 1950s as a result of the combination of the techniques of density sampling, using an array of particle detectors to find the shower centre of symmetry¹ and fast timing using spaced scintillation counters to fix the shower arrival direction². Previous Cerenkov light measurements in extensive air showers have concentrated on the gross properties of the light flux on the ground using a similar approach to the particle measurements. It has been suggested by Fomin and Khristiansen³ and Efimov *et al.*⁴ that the full-width half maximum (FWHM) of pulses of Cerenkov light may depend on the longitudinal development of the shower, and so on the atomic mass number of the primary. We have gone further and used precise measurements of the detailed shape of the Cerenkov light pulses recorded by several well spread simple detectors to reconstruct accurately an image of the shower in Cerenkov light. This has been done without the usual prerequisite of first determining the shower's axis of symmetry and arrival direction. The data so obtained may be of use in studies of the average shower characteristics, the search for extreme

fluctuations and, we hope, for the labelling of individual showers with an indication of the mass of their primary.

Measurements were made during about one hundred hours of clear sky, moonless night-time during winter 1975-76 with an array of eight Cerenkov light detectors spread over an area of 1 km² at Haverah Park near Harrogate. Each detector comprised a fast photomultiplier with 5 inch diameter photocathode (RCA type 4522) viewing the night sky directly; the pulses were recorded using a cable delay and oscilloscope display system with a 40 MHz bandwidth. The results to be discussed here are based upon a limited sample of showers (primary energy >10¹⁷ eV) in which at least six detectors recorded Cerenkov light pulses at distances 100-600 m from the shower core. The time of occurrence of light at the 10%, 50% and 90% of full pulse height levels was measured in the rising and falling edges of the pulse to an accuracy of ±2-3 ns from the film records.

The likely origin in the atmosphere of the light observed at various times in the pulse has been made clear by computer simulations. For example, the light pulse at 300 m from the core of a 10¹⁷ eV proton initiated shower is shown in Fig. 1 together with the electron cascade development (Protheroe and K.E.T., unpublished observations). This electron cascade is divided into eight sub-showers and the corresponding eight sub-pulses of Cerenkov

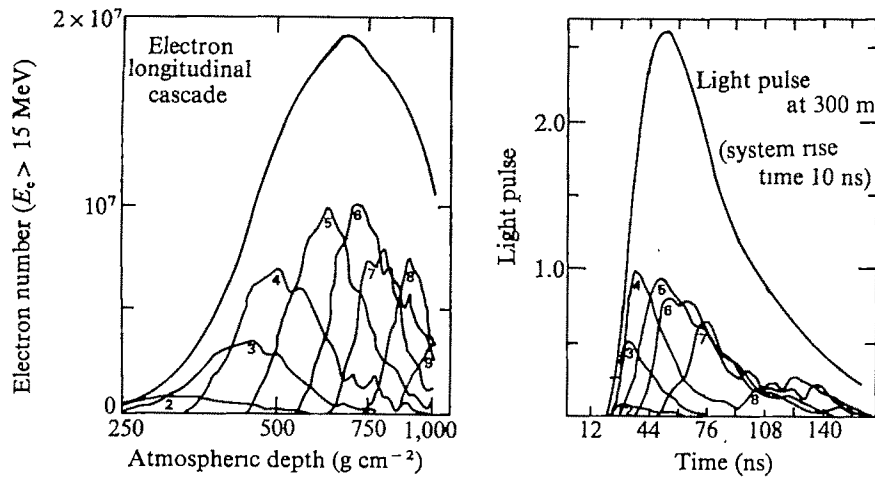


Fig. 1 The longitudinal electron cascade and light pulse shape at 300 m from the core of an average 10^{17} eV proton initiated shower. The light sub-pulses arising from eight sub-showers indicate the origin in this electron cascade of the light in different parts of the light pulse.

light are indicated. It is clear that despite refractive index effects, the earliest observed light originates high in the atmosphere and the pulse shape is clearly a direct measure of the cascade development.

On the assumption that the light at a certain point in each pulse originated from a point in the atmosphere, least square fits of spheres to the sets of times at which the 10% full height was achieved were attempted. These times were found to be very well represented by a sphere (r.m.s. deviation of 3 ns from fronts of radius approximately 7 km (20,000 ns)) and the coordinates of the origin were determined to an accuracy of about 100 m.

The fitting procedure was repeated for the times of the light at the 50 and 90% levels in the rising and falling edges and the origin heights of typical near vertical showers are shown in Fig. 2, after conversion to atmospheric depths in g cm^{-2} . These data show the development of two individual showers in Cerenkov radiation through the atmosphere.

The average measured development of the image of showers of energy about 3×10^{17} eV in Cerenkov light is shown in Fig. 3 together with directly comparable simulation data. The computer simulations were based upon a detailed treatment of air Cerenkov light seen by detectors of the type and bandwidth used in our experiment according to an air shower model incorporating contemporary hadron physics (R. J. Protheroe, G. J. Smith and K.E.T., unpublished). The sensitivity of this technique is clearly shown by the fact that the data of Fig. 3 are based upon showers recorded with zenith angles up to 40° which have very different measured heights of origin (km) but similar depths of origin (g cm^{-2}).

There is no requirement in our analysis of the arrival direction for the centre of symmetry (the core) of the shower, since the spherical light fronts are deduced

directly from the measured pulse shapes and give unambiguously the coordinates of the origin of the light. However, the path traced through these points of origin of light in an individual shower corresponds to the trajectory of the shower core through the atmosphere and provides an accurate measure of the arrival direction (typical errors in angle of arrival are $< 0.1^\circ$). Furthermore, the intersection of this trajectory with the ground plane gives the core impact location (which forms the basis of conventional analyses). Agreement between this impact point and the conventional core location from particle density or Cerenkov light detector arrays is better than

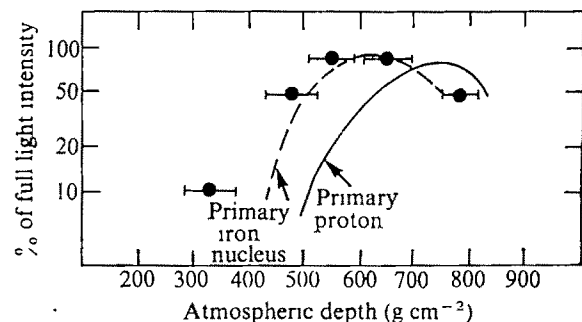
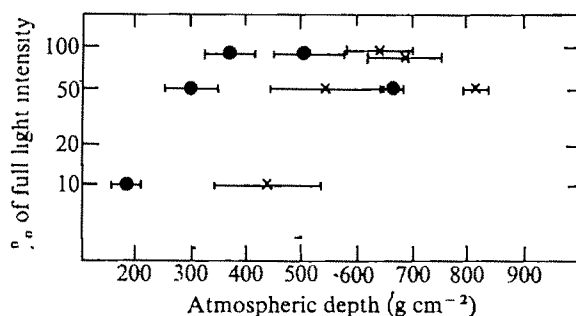


Fig. 3 The average Cerenkov light image of the cascade development of showers (primary energy about 2×10^{17} eV). The broken and solid lines indicate the computed average Cerenkov images for iron nucleus and proton induced showers of comparable energy derived using a shower model incorporating scaling.

Fig. 2 Cerenkov light images of the cascade development through the atmosphere of two showers (primary energy about 10^{17} eV).



50 m in well measured showers. The information available from this technique is summarised schematically in Fig. 4.

It is now possible to rank showers in primary energy in the conventional way on the basis of a photon density at a prescribed distance from the core (simulations indicate a preferred distance of 200–300 m). However, we are also able to rank showers in primary energy from Cerenkov light data without the requirement to know the core position at ground level. This arises since each Cerenkov light detector measures the total light pulse area (which depends strongly upon primary energy and less so upon the individual cascade development, distance from the core of the detector and zenith angle and so on), and the pulse FWHM (which is nearly independent of primary energy and cascade development and varies only with core distance and zenith angle). We regress the values of the pulse area and their corresponding values of the FWHM and obtain that value of the area corresponding to a FWHM of 40 ns. (This corresponds in a conventional

analysis to the pulse area at a core distance of about 300 m.) There is good agreement between this quantity and the conventional measure of the primary energy—the optical density at a distance of 200 m or the Haverah Park ground parameter $\rho(500)_{VB}$ measured by the University of Leeds group—but it emerges without any of the usual core fitting analysis procedures.

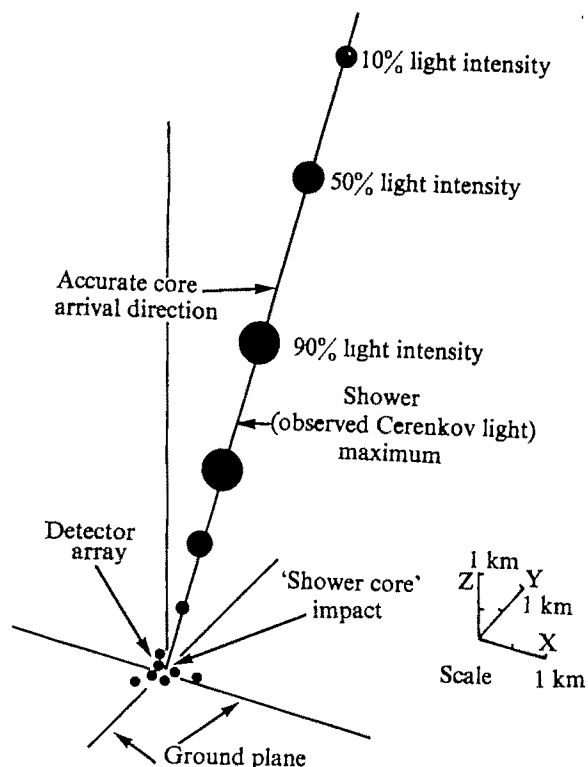


Fig. 4 A Summary of the information available on individual showers from this interpretation.

The main source of error in our present measurement which was made with preliminary equipment is in the time of the beginning of each pulse (the 10% of full light level) relative to the other pulses (r.m.s. error=3.7 ns corresponding to uncertainties of about 100 g cm^{-2} in the initiation depths), the individual pulse shapes are internally well measured (r.m.s. error=1.4 ns) and so the shapes of the inferred shower cascades are at present more reliable than the point of initiation of the shower.

We suggest that a simple, novel and economic method has been demonstrated based upon Cerenkov light measurements for the accurate determination of arrival direction, the estimation of primary energy and, the most important of all, direct observation of the cascade development in individual showers. This method does not depend upon the usual approach to shower analysis and does not require an accurate centre of symmetry (shower core) be determined from ground level data. We consider that measurements of the type described here, employing our improved equipment at locations enjoying long periods of clear sky conditions, may contribute substantially to attempts to measure the atomic mass number of the primary cosmic rays.

We are grateful for the assistance in the operation of the array and the analysis of the data given by Mr R. T. Hammond and Mr D. W. Wellby. The development of this technique was assisted by the availability of the analysis of the Haverah Park particle detector array data

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Possibility of using ^{81}Kr to detect solar neutrinos

THE state of the experiment designed to detect solar neutrinos by the reaction $\nu + ^{37}\text{Cl} \rightarrow ^{37}\text{Ar} (T_{1/2} = 35\text{d}) + e^-$ (threshold = 814 keV) has been summarised by Bahcall and Davis¹, who report essentially a null result for the average of many runs over the past few years. The experiment is sensitive only to a tiny fraction of the total flux, that is to neutrinos arising from the ^8B decay (Table 1) and it is therefore essential to have another experiment which responds to the lower energy, more abundant neutrinos if the fundamental ideas about the mechanisms by which the Sun produces its energy are to be tested. There seem to be very few^{2,3} experiments with this property and an interesting alternative approach has been suggested by Freedman *et al.*³. They hope to obtain a value of the average flux during the past few Myr by measuring the ^{205}Pb produced in old, thallium-rich minerals in the reaction $\nu + ^{205}\text{Tl} \rightarrow ^{205}\text{Pb} (T_{1/2} = 1.6 \times 10^7 \text{ y}) + e^-$ (threshold = 46 keV), which is sensitive to all parts of the neutrino-producing chain. The purpose of this communication is to investigate the feasibility of detecting solar neutrinos through the reaction $\nu + ^{81}\text{Br} \rightarrow ^{81}\text{Kr} (T_{1/2} = 2.1 \times 10^6 \text{ y}) + e^-$ (threshold = 490 keV), which turns out to be sensitive mostly to the ^7Be neutrinos, the second most abundant in the chain.

The requirements for any such "geological" experiment are that the interacting material has been sufficiently deeply buried that the neutrino effect is not swamped by cosmic-ray interactions, that the cover has been in place long enough for any previously produced ^{81}Kr to decay, that the cross-section for neutrino interactions be calculable and that there is sufficient material available. Bromine tends to be a trace element in nature and the chances of obtaining sufficient quantities (estimated at a few hundred kg) of a Br-rich mineral such as AgBr from a great enough depth seem to be low. Certain salt deposits, however, offer a possibility. These are old (Permian, 240 Myr), can be deep (> 1,000 m) and the later crystallisations of such evaporate deposits tend to concentrate Br relative to Cl. For example, in carnallite ($\text{KCl MgCl}_2 \cdot 6\text{H}_2\text{O}$) the ratio of Br:Cl can be 0.01:1 by weight⁴. This mineral is found in large quantities in the Stassfurt deposits and the amount required for a viable experiment can be estimated as follows.

With the above Br:Cl concentration there will be 1.42×10^{19} ^{81}Br atoms per g of carnallite and at saturation (that is after a few

Table 1 Values of the cross-section and interaction rate for the reaction $\nu + ^{81}\text{Br} \rightarrow ^{81}\text{Kr} + e^-$ calculated as described in the text

Reaction	E_ν (MeV)	ϕ ($\text{cm}^{-2} \text{s}^{-1}$)	σ (10^{-48} cm^2)	$\sigma\phi$ (SNU)
$p + p \rightarrow d + e^+ + \nu$	0-0.42	6.1×10^{10}	0	0
$p + e^- + p \rightarrow d + \nu$	1.44	1.5×10^8	3.02	0.45
$^7\text{Be} + e^- \rightarrow ^7\text{Li} + \nu$	0.86	3.4×10^9	1.06	3.60
$^{13}\text{N} \rightarrow ^{13}\text{C} + e^+ + \nu$	0-1.2	2.6×10^8	0.74	0.19
$^{16}\text{O} \rightarrow ^{16}\text{N} + e^+ + \nu$	0-1.74	1.8×10^8	1.50	0.27
$^8\text{B} \rightarrow ^8\text{Be}^* + e^+ + \nu$	0-14	3.2×10^9	61	0.20

Myr) the number of ^{81}Kr atoms per g will be $1.36 \times 10^{34} \sigma \phi$, where σ is the neutrino interaction cross-section and ϕ the neutrino flux. $\sigma \phi$ is conventionally quoted in solar neutrino units (SNU) where $1 \text{ SNU} = 10^{-36}$ interactions per nucleus per s. The flux can be calculated from solar models⁵ and the cross-section obtained from the relations given by Bahcall⁶. Thus for a neutrino-induced reaction which produces an electron in a continuum state of total energy W and momentum p , and in which the neutrino is monoenergetic,

$$\sigma = \frac{2\pi^2 \ln 2 p W F(Z, W)}{ft} \left(\frac{\hbar^2}{m^2 c^2} \right)$$

where $F(Z, W)$ is a tabulated function giving the effect of the nuclear coulomb field on the electron, ft is the comparative lifetime of the reverse, electron capture decay and m is the electron mass.

The ^{81}Kr $7+1/2$ ground state electron capture decay to the $3-1/2$ ground state of ^{81}Br ($Q_{\text{e.c.}} = 300 \text{ keV}$) has $\log ft = 11.6$ and the reverse, neutrino-induced reaction has a very small cross-section. There is, however, a $1-1/2$ isomeric state ($T_{1/2} = 13 \text{ s}$) of ^{81}Kr at 190 keV excitation and this is the state which would be populated by the neutrino reaction. The electron capture branch of this state has not, unfortunately, been observed (the branching relative to γ emission can be roughly estimated at one in 10^5) and it is necessary to estimate the ft value from the systematics of similar decays in this region, which is an awkward part of the periodic table as far as nuclear theory is concerned because there are several nucleons outside closed shells and a complicated structure of excited states. The following $\log ft$ values emerge: ^{79}As ($3-1/2$, ground) \rightarrow ^{79}Se ($1-1/2$, isomer): 5.3; ^{81}As ($3-1/2$, ground) \rightarrow ^{81}Se ($1-1/2$, ground): 5.2; ^{81}Rb ($3-1/2$, ground) \rightarrow ^{81}Kr ($1-1/2$, isomer): 5.1; ^{83}Br ($3-1/2$, ground) \rightarrow ^{83}Kr ($1-1/2$, isomer): 5.0. We adopt a value of $\log ft = 5.3$ in the calculation and thus obtain $\sigma = 0.5 \times 10^{-45} W (\text{MeV}) p (\text{MeV}/c) F(Z, W) \text{ cm}^2$. (The restriction to $3/2 \rightarrow 1/2$ transitions ensures that there are no spin-dependent statistical factors arising from considerations of detailed balance.) The various neutrino-producing reactions are shown in Table 1 with the fluxes (unpublished data of M. S. Freedman *et al.*) and cross-sections calculated from the above formula. In the case of the reactions producing a spectrum of neutrino energies, the average value of the continuous spectrum was used in the calculation. This does not introduce much error because most of the interactions are with the monoenergetic ^7Be neutrinos.

The total interaction rate of 4.7 SNU would yield 6.4×10^{-4} atoms of ^{81}Kr per g of carnallite at saturation. Thus a technically feasible experiment might use 100 tonnes of carnallite dissolved in water (giving $1.5 \times 10^5 \text{ l}$ of solution, similar in quantity to the $4 \times 10^5 \text{ l}$ of C_2Cl_4 used in the Davis experiment) with the 6.4×10^4 atoms of ^{81}Kr being extracted by methods similar to those used by Davis¹. This number of atoms corresponds to a volume of $2.4 \times 10^{-16} \text{ ml}$ of ^{81}Kr at STP, which is about the limit of detection by mass spectrometry in favourable cases⁷. Whether the present case is favourable or not depends largely on the amount of atmospheric Kr which was dissolved in the sea at the time the salt deposits were being laid down and what happened to it thereafter. The Kr content of evaporites does not seem to have been measured but there are some results for Ar (ref. 8) and if these are relevant to Kr it would seem that interference from the scattering tail of ^{81}Kr could be the main problem in the mass spectrometry, for the atom ratio of $^{81}\text{K} : ^{81}\text{Kr}$ might be as low as $1:10^{13}$ (personal communication from C. M. Stevens). There is also the question of whether or not the ^{81}Kr produced in the atmosphere by cosmic radiation might be introduced into the mineral. The possibility of escape of Kr during any recrystallisation could be estimated by performing a K-Ar age determination on the mineral and assuming that Kr and Ar behave in a similar way.

To establish whether or not this experiment is possible it is necessary to have a measurement of the ^{81}Kr content of

carnallite, some more information about the availability of suitable quantities of Br and either a better theoretical estimate of the ft value or a measurement of the decay branching. The first measurement is presumably straightforward and would determine whether the idea of carnallite is worth pursuing. If it is not, then the experiment could still be done if Br were available in some other form in which the problem of ^{81}Kr was not so severe. The best hope of an improved ft value probably lies in a search for the electron capture branch of the isomeric decay, using the best resolution Si (Li) detector available to look for Br K X-rays in the presence of a very much greater number ($\sim \times 10^5$) of Kr K X-rays, produced after internal conversion of the 190-keV γ ray. It might turn out, on the basis of the results of the investigations suggested above, that the experiment is impossible—even if all other considerations were favourable, for example, a $\log ft > 6$ would probably make it so. If the solar neutrino problem is to be solved, however, it will probably be necessary to consider any possibility of a viable experiment, no matter how impractical it might seem at first. There are no easy solar neutrino experiments and, almost certainly, they will all be conducted at the outer limits of the techniques involved.

I thank Drs M. S. Freedman and C. M. Stevens, of the Argonne National Laboratory, for arousing my interest in this matter and for helpful correspondence. I thank the staff of the Isotope Geology Unit at SURRC for discussions.

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Prediction of coronal structure of the solar eclipse of October 23, 1976

PREDICTION of solar eclipse coronal structures began in 1968¹ as an offshoot from suggestions by Chapman² and Gold³ that the magnetic field in the vicinity of the Sun could be calculated. A degree of success was achieved with these early attempts^{4–6}. Cowling⁷ states that “a sketch showing the corona observed at the eclipse . . . agrees well with the prediction; had Schatten drawn his streamers more nearly radial, the agreement would have been almost perfect”.

This disagreement in the direction of the streamers in the outer corona with those observed arose from the early model's^{8,9} inability to calculate field directions beyond where the solar wind carried out the extended solar magnetic field, approximately one solar radius above the photosphere. A new “current sheet” model¹⁰ has been developed which allows precisely this calculation to be performed. The mathematics and physics of this model are rather complex. Basically, however, the model utilises a current-free inner corona ($1 R_{\odot} < r \leq 1.6 R_{\odot}$), with fields calculated from Legendre polynomials using potential theory fitted to observations of the photospheric line-of-sight magnetic field taken at the Hale Observatories at Mount Wilson. In the outer corona ($r > 1.6 R_{\odot}$), thin, force-free current sheets “open” the magnetic field to the solar wind. They allow the magnetic stress tensor to be balanced everywhere in the outer corona and represent a best Legendre polynomial fit to the previously calculated field at 1.6 solar radii. Force-free current sheets have been shown¹⁰ to resemble closely calculations of isothermal coronas for dipole solar fields¹¹.

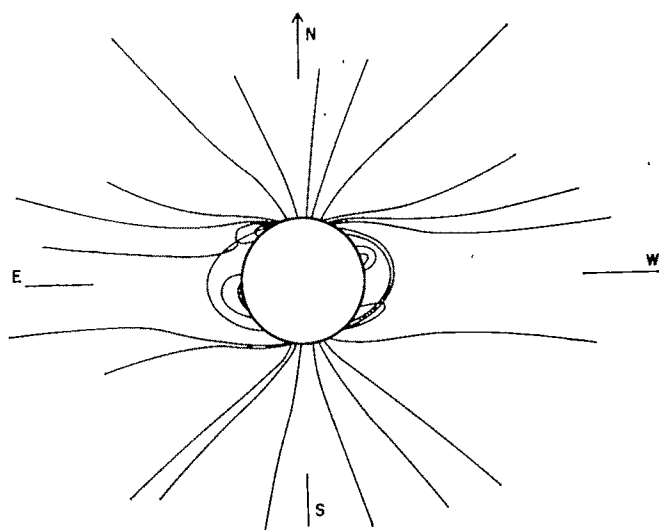


Fig. 1 Sketch of the corona based upon the computer drawn map of the coronal field patterns. The brightest feature of the corona should be the two large equatorial streamers.

The use of force-free current sheets is supported theoretically by the relative weakness of particle pressure compared with magnetic stresses in the outer corona below 20 solar radii⁸.

The photospheric magnetic field data utilised in predicting the eclipse were obtained during Carrington solar rotation 1646. This solar rotation runs from September 13.63 to October 10.90. Thus the east limb data (longitude 104°) are approximately 20 d old on October 23, whereas the west limb data (longitude 284°) are approximately 34 d old on the day of the solar eclipse. Due to the low level of solar activity at present it is thought unlikely that the corona will be seriously disrupted before the eclipse by a new large solar active region.

Before the data could be utilised in the Legendre fit, a considerable amount of preprocessing was necessary at the Hale Observatories. Thus the west limb data are one rotation older at eclipse time than the data during the predictions made in 1968 and 1970. This disadvantage is offset by the considerable advantage gained from having full coverage at all latitudes (except for five degrees near the southern pole of the Sun which are continually tilted away from the Earth at this time). In 1968 and 1970, data equator-ward of 40° latitude were utilised plus mean north and south polar field values. With the digitisation of the data, full polar coverage is now possible.

A computer drawn plot made on October 18 showed the field line structure predicted for the time of the solar eclipse. Figure 1 is a drawing based upon this plot outlining the main features. As can be seen, a very dipolar coronal field results. One huge equatorial streamer is predicted for both the east and west limbs of the Sun. This is due to the lack of very strong active regions near either limb of the Sun. However, nested coronal arches may be seen within this equatorial streamer, below 0.6 solar radii above the limb. Many small arches at latitudes below 60° may be seen on either limb. They are so numerous, they may obscure each other. In particular, on the west limb low arches between 42° and 48° North should appear and on the east limb, low arches near 50° North. In addition, a new active region on the west limb should make its presence known by low arches, and possibly a streamer, near south 26° latitude. However, the main feature of this eclipse should be the two large bright streamers marking the solar equator, with polar plumes in a characteristic dipole fashion.

This total solar eclipse, weather permitting, will be visible over the Indian Ocean, south Australia, and the south Pacific, north of New Zealand. The largest city where this eclipse may be seen will be Melbourne. A number of observers will be there and it is hoped a high resolution photograph with a radial

density filter will allow much of the structure to be discerned for later comparison.

I thank Drs Robert Howard and John Adkins of the Hale Observatories for making available their photospheric magnetic field data. This research was supported by the National Science Foundation, Atmospheric Research Section. I thank Mr Mark Bernstein for his help in handling the Unicol computer which performed the calculations. A copy of the program which performed these calculations can be obtained from the National Space Science Data Center Code 601 GSFC, Greenbelt, Maryland 20771 by asking for the Quiet Sun Magnetic Field Program.

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Another correlated X-ray-radio transition in Cygnus X-1

In this letter we report that in February 1976, the X-ray and radio emission from Cyg X-1 underwent a simultaneous transition, similar to that observed in March 1971 when the source was first detected at radio frequencies.

The Cyg X-1 region was observed with the Westerbork synthesis radio telescope at 1,415 MHz on four occasions in February–March 1976. Each observation lasted for twelve hours, resulting in an r.m.s. noise of 0.4 mJy. Comparison of the four measurements showed no significant changes in the flux densities of the sources in the field, except for Cyg X-1. The results are shown in Table 1 and Fig. 1.

On February 13, the Cyg X-1 radio source was at a level considerably lower than the lowest level observed since the well known March 1971 transition^{1–6}. Between February 13 and March 6, its intensity rose sharply, peaked, and apparently levelled out close to its usual value of 15 mJy. This behaviour is remarkably similar to that seen in 1971.

Also as in 1971⁷, the dramatic increase in radio intensity was accompanied by a decrease in X-ray intensity by about the same factor⁸. This X-ray transition is shown schematically in Fig. 1. Before the transition, the source had been in its “high” X-ray state since November 1975. It was also in that state for a period of months before the March 1971 transition⁷, and apart from these two occasions, no other instances of such a persistently high emission level have ever been recorded for it⁹.

Between March 1971 and November 1975, the source was in its “low” X-ray state and its radio emission was remarkably constant. The only exception was during May 1975^{5,6}.

Table 1 1,415 MHz flux densities of Cyg X-1

Universal time 1976	S_{1415} (mJy*)
February 13 ^h 03 ^m 59 ^s –13 ^h 16 ^m 01 ^s	5.0±0.6
February 22 03 24 –22 15 26	18.5±1.0
February 27 03 04 –27 15 06	23.1±1.2
March 06 02 33 –06 14 35	18.3±1.0

*1 mJy = 10^{-26} W m⁻² Hz⁻¹

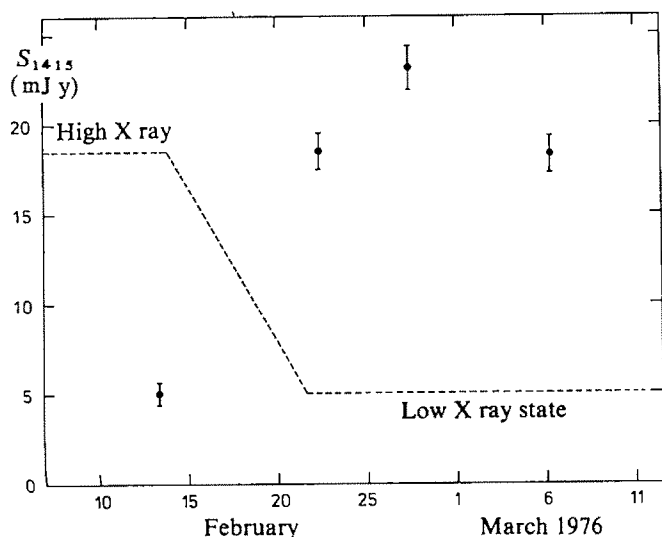


Fig. 1 The radio and X-ray behaviour of Cyg X-1 during February–March 1976. The radio data are from Table 1. The dashed line gives a schematic representation of the Ariel V X-ray data taken from Holt *et al.*⁸.

At that time, the X-ray and radio emission both increased, distinguishing that short lived “transient event” from the “transition” reported here.

There are several possible explanations for the anti-correlation observed in the X-ray and radio behaviour of Cyg X-1 during transitions. First, heightened X-ray emission could be produced by a hot gas which eventually envelops the radio emitting region, blanketing it out by free-free absorption. When the gas expands sufficiently, the cut-off frequency would become lower and the radio source would reappear in its normal state. Second, the event responsible for the enhanced X radiation might either interrupt the supply of relativistic electrons to a non-thermal radio source or result in such an increased radiation density that inverse Compton losses would extinguish the source. A cut-off of the additional X-ray supply would then again result in a return of the radio source to its normal state. To decide between these and other alternatives, it is crucially important to monitor the radio spectrum of Cyg X-1 throughout future transitions.

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New test of the cosmological nature of QSO redshifts

In spite of extensive observations the nature of the redshift of a quasistellar object (QSO) remains a controversial issue¹. The redshift–magnitude relation, QSO–galaxy associations, time variability faster than light motion and several other criteria are being used to argue in favour of or against the cosmological nature of the QSO redshift. It is therefore desirable not only to improve the statistics of the existing data but also to think of new tests to clarify this important issue. Recent improvements^{2,3} in the spectrophotometry of QSOs enable us to propose a potential test of the cosmological hypothesis in the following manner.

Consider a plot of the emission line width (for example, the full width at half maximum intensity, FWHM) of a specified spectral line against the redshift of a QSO. Assuming that all QSOs are intrinsically identical, and are at cosmological distances corresponding to their redshifts, the FWHM of a typical line of rest wavelength λ_0 in a QSO of redshift z is given by

$$w = w_0(1+z) \quad (1)$$

where w_0 is the FWHM in the rest frame of the QSO. A plot of w against $1+z$ should therefore be a straight line through the origin. The test involves plotting w against $1+z$ for a large number of QSOs, using the same spectral line for all of them.

In a hypothesis where the redshift is largely of gravitational origin there is no simple model-independent relation like equation (1). For example, in the model of the type considered by Greenstein and Schmidt⁴, the line width arises from the gravitational broadening at the surface and the relation between w and z is given by

$$w = \frac{R_g}{2R} \lambda_0 (1+z)^3 \frac{\Delta R}{R} \quad (2)$$

where R_g is the gravitational radius of the object, $4\pi R^2$ is the surface area of the object and ΔR the thickness of the emitting region in the Schwarzschild coordinates. Both R and ΔR depend upon the model. For the Hoyle–Fowler type models with central emission line redshifts⁵ even more complicated forms than equation (2) arise.

For the local Doppler shift explanation of the type advanced by Terrell⁶ the relation (1) will hold. Thus a tight straight-line plot will be a positive test of the cosmological or Doppler hypothesis. In practice, however, the test is not so easy to apply and we discuss briefly some of the difficulties and how they could be resolved.

First, the measurements of FWHM have to be made very accurately. With the image tube scanner technique⁷ it is possible to have resolutions better than 7 Å in FWHM. Since the line widths are at least 20 Å in most cases it now seems possible for at least a start to be made. It is of course necessary to choose the same spectral line for all QSOs in any given sample. Thus Ly α may be chosen for high redshift QSOs ($z \gtrsim 1$) and the MgII line for low redshift QSOs ($z \lesssim 1$). The CIV line can also serve a good line for a large number of QSOs of moderately high redshifts.

A preliminary ($w, 1+z$) plot for a sample of southern sources^{7,8} reveals a large scatter (see Fig. 1) for the MgII line of low redshift QSOs. A similar plot for the CIV line in a number of high redshift QSOs based on the data given by J. Baldwin (personal communication) also shows no linear relationship. The scatter may of course be due to variation in w_0 from QSO to QSO, and this illustrates another difficulty of arriving at a decisive conclusion. However, intrinsic line widths probably do not vary by more than a factor of 10 and the scatter could be reduced by properly selected criteria, for example the nature of radio spectra⁹. To arrive at a suitable criterion it is necessary to have a better understanding of the causes of emission lines

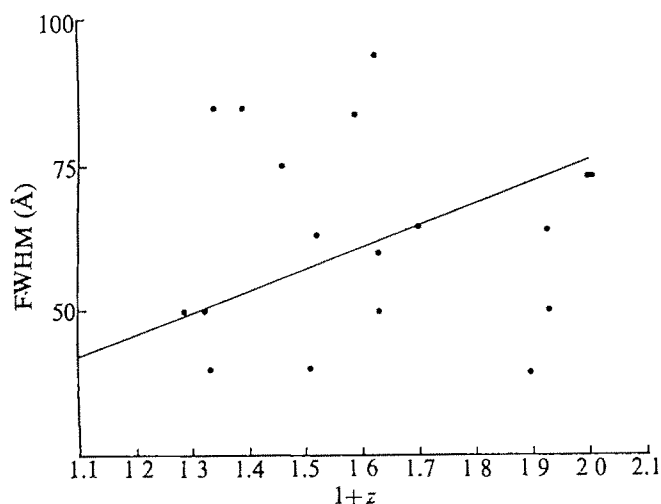


Fig. 1 Plot of FWHM against $1+z$ for MgII line for 18 southern sources. The scatter about the line of least square fit to relation (1) shows how at present no linear trend is apparent from the data.

in QSOs from theoretical as well as from empirical approaches based on observations¹⁰. The situation is no worse than in the $m-z$ relation for QSOs, where the scatter is supposed to arise (if the cosmological hypothesis is right) from the variation in the intrinsic luminosity by several magnitudes. On the other hand the proposed test is free of the added uncertainty of not knowing the deceleration parameter q_0 in the $m-z$ relation.

It is possible to apply this test to emission line galaxies such as Seyferts, but the smallness of the redshift makes the variation of w with z insensitive according to equation (1). For QSOs, with their large redshifts, this test appears better suited. With the redshifts and extensive optical information on more than 570 QSOs available (Burbidge, Crowne and Smith, preprint) it may be worthwhile attempting this test with better measurements of line widths which should become possible in the future.

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Interacting galaxies and QSO absorption lines

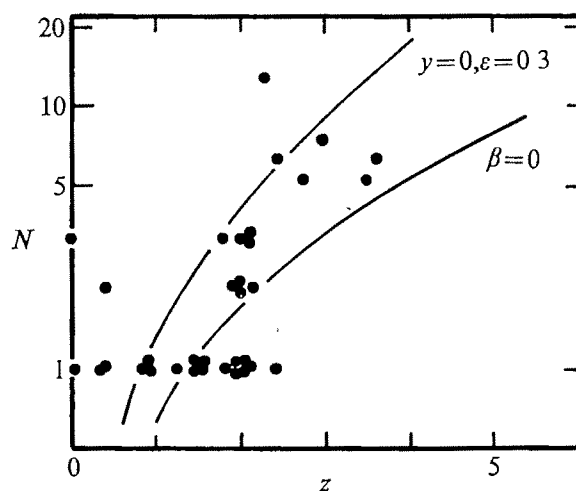
THE spectra of quasi-stellar objects are observed to have absorption lines with redshifts mostly below the redshift of the emission lines¹. The absorbing clouds are thought to be either near the QSO¹⁻⁵ or at cosmological distances from it⁶⁻⁸. On the first interpretation, the clouds are supposed to be accelerated to high velocities ($\lesssim 0.5c$ away from the QSO) by radiation pressure.

However, the small internal velocity dispersions inferred for the clouds from the absorption line widths ($\sim 50 \text{ km s}^{-1}$) pose serious problems to this model⁹ and we consider here the second interpretation. The interception cross section of intervening galaxies for QSO photons has hitherto been calculated¹⁰ on the assumption that the galaxies do not evolve. The data so far available (Fig. 1), although probably contaminated by selection effects¹, seem to indicate that the number of absorption redshift systems increases more rapidly as a function of the emission line redshift than can be accounted for without such evolution. The possibility has been considered^{7,8} that the intervening clouds are collapsing protogalaxies, but we think it likely that most galaxies form¹¹ before $z=5$ and, instead, attribute the rapid increase in the number of redshift systems to tidal interaction of galaxies at earlier epochs. Because galaxies were closer together in the past, chance collisions were more frequent and the effects of these interactions were more pronounced.

The effects of an encounter between a galaxy and a neighbour are proportional to the tidal force and to the duration of the encounter. This force is inversely proportional to the cube of the distance r between the galaxies, the duration of the collision is proportional to r/v , where v is the relative velocity of the galaxies (since we intend to calculate the probability of a tidally distorting collision as a function of z only, the vectorial properties of the collision are irrelevant). Thus, the amount of disruption that a galaxy suffers in an encounter is proportional to $r^{-2}v^{-1}$. The expectation value of this quantity can be calculated if we know the probability of finding, near any given galaxy, another one with distance between r and $r+dr$ and with velocity between v and $v+dv$. In other words, we must know how the phase space spanned by r and v is occupied and then convolve $r^{-2}v^{-1}$ with the density distribution in phase space.

The average distribution is found from the cosmological model used to describe the large-scale evolution of the Universe, but in addition the effects of clustering must be considered. The empirical evidence on the pair correlation of galaxies¹² is unfortunately insufficient for our purposes, first because the dependence of the correlation on redshift is unknown, second because the velocity correlation is unknown. We are currently working on the results of an N -body model of the evolution of clustering to determine the density distribution in phase space and here anticipate the results by assuming the Keplerian approximation $v \propto r^{-1/2}$ and by assuming that the clustering at

Fig. 1 Number N of absorption redshift systems against the emission redshift of the background QSO. Dots: observations¹, drawn lines: predictions for a universe with $q_0 = 0$. Lower curve: no tidal distortion. Upper curve: tidal distortion parameter $\epsilon = 0.3$, clustering parameter $\gamma = 0$. Because of incompleteness of the sample, N has not been expressed as a fraction of the total number of sources observed. Therefore, the observed points may be fitted to the curves by applying an unknown multiplication factor to N , which in the diagram corresponds to vertical displacement.



redshift z can be described by the usual linear perturbation of the cosmic number density,

$$n = n_0(1+z)^3[1 + (1+y)/(1+z)] \quad (1)$$

where y is the redshift at which the density perturbation reaches unity.

Under these assumptions, the expectation value of the factor β by which the projected area of a galaxy increases due to an encounter is $\beta \propto r^{-2} v^{-1} \propto r^{-3/2}$, and because $r \propto n^{-1/3}$ we obtain

$$\beta = \varepsilon(n(z)/n_0)^{1/2} = \varepsilon(1+z)(2+y+z)^{1/2} \quad (2)$$

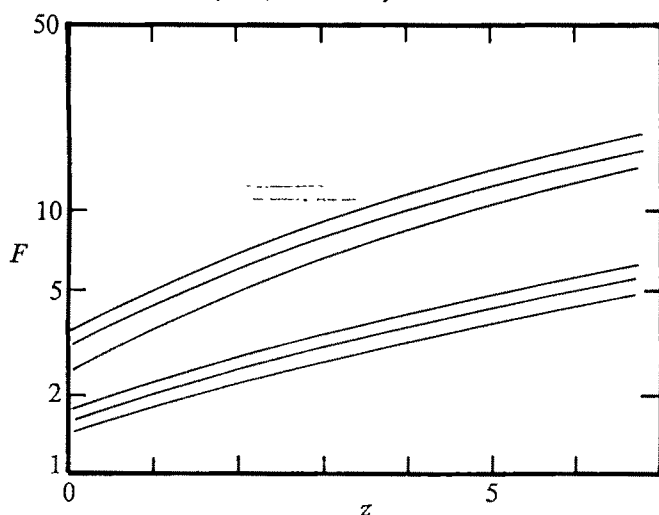
The parameter ε is estimated by noting that numerical simulations¹³ give at least a fivefold area increase for a collision with a periastris of a few galactic radii. Thus, if the mean distance between galaxies with a mass of 10^{41} kg is 1.5 Mpc, one has $\varepsilon \approx 5(0.1/1.5)^{3/2} = 0.07$. Observations of the interacting system M81/M82 show¹⁴ a hydrogen envelope having a projected surface area of about 10 times the combined Holmberg areas of the individual galaxies. This suggests $\varepsilon \approx 0.2$, but the collision may be atypical. With equation (2), the probability P that the line of sight from us to a QSO with redshift z cuts a galaxy or a tidal extension is given by

$$P \propto \int_0^z (1+z')(1+\beta(z'))dz' \\ = \left[\frac{1}{2}(1+z')^2 + \varepsilon \frac{2}{3}(2+y+z')^{3/2} \left\{ (1+z')^2 - \frac{4}{3}(1+z')(2+y+z') + \frac{8}{33}(2+y+z')^2 \right\} \right]_0^z \quad (3)$$

It has been assumed that $q_0 = 0$, which avoids complicated functions in (3) and is for the present purposes sufficiently near the value $q_0 = 0.05$ that corresponds to the mean cosmic density implied above.

In Fig. 1 we have drawn the function (3) for $\varepsilon = 0$ (see ref. 10) and in Fig. 2 the factor with which this must be multiplied to obtain the solution for various values of ε and y . This factor equals the relative proportion of lines due to bridges and tails of all absorption line systems. The optical depth¹⁰ of the lines in absorption systems due to a bridge or a tail will be smaller than those due to galaxies proper. The ionisation state of the tidal extensions could be caused by the higher ultraviolet background radiation expected^{7,8} at higher values of z . As appears from the

Fig. 2 Factor F with which the number of intercepting galaxies expected for $q_0 = 0$ (see text) must be multiplied in order to obtain the number expected when tidal interaction is taken into account. Upper triple: tidal distortion parameter $\varepsilon = 1$, lower triple $\varepsilon = 0.3$. Of each triple, the upper curve has clustering parameter $y = 4$, middle curve $y = 2$, lower curve $y = 0$.



figure, tidal extensions could contribute significantly to the interception cross section of galaxies at large redshifts.

Predictions of our model are (1) because the probability of tidal deformation increases with z , the fraction of QSOs with shallow absorption lines also increases with z , (2) the abundances of the absorbing atoms are typical for the interstellar medium: (3) absorption line splitting of the order of a few hundred kilometers per second (that is, the velocity at periastris of slightly hyperbolic orbits) is possible, but the splitting should not be 'magic'. Observational tests on the basis of existing data are not yet possible for (1), confirm^{1,2} (2) and are inconclusive¹⁵ for (3). We feel that the third one is the acid test: it is, of course, a key prediction of our model that the absorption line splitting will not be constrained to certain 'magical' intervals in frequency.

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Theoretical maximum for energy from direct and diffuse sunlight

SCHEMES for the conversion of sunlight to useful (electrical, mechanical, chemical) energy all make use of the high spectral temperature of solar radiation relative to the terrestrial ambience. Some schemes, but not others, also make use of the high directivity of the solar flux. For example, focusing mirror arrays require direct sunlight, while photovoltaic devices are indifferent to the directness or diffuseness of light of a given intensity. In biological systems, photosynthesis evidently makes little use of sunlight directivity, since it is not observed to depend strongly on plant orientation on angular scales as small as half a degree (the size of the Sun); on the other hand, Kevan¹ reports some heliotropic, arctic flowers whose corollas are nearly paraboloidal and focus direct (but not diffuse) radiation on the sporophylls.

The maximum thermodynamic efficiency permitted for extracting energy from direct sunlight must be higher than that permitted for diffuse sunlight of the same intensity, since loss of directivity is evidently an irreversible process. But what are the numerical values of these two maximum efficiencies? Are they different enough to justify *a priori* a concentration of effort by man or plant on the exploitation of direct solar radiation only? These are the questions to be considered briefly here, somewhat more specifically than can be found in the literature^{2,3}. The answers are in some ways surprising. For example, neither direct nor diffuse light yields the efficiency formula $(T_{\odot} - T_{\oplus})/T_{\odot}$ that

one might naively expect (where T_{\odot} is the equivalent black-body solar temperature, $\sim 5,800$ K, T_e is the ambient temperature on Earth, ~ 300 K).

We take the input thermodynamic system to be a volume sample of the ambient radiation field at the Earth's surface, including fluxes from the Sun, sky and ambient surroundings. Per unit volume suppose that this radiation field has energy E and entropy S . Since a black-body (maximum entropy) distribution has an energy density $a(S/(4/3)a)^{4/3}$, an amount of work equal to $E - a(S/(4/3)a)^{4/3}$ can be extracted isentropically, leaving a black-body distribution of temperature $T_{\star} = (S/(4/3)a)^{1/3}$. (Here a is the usual radiation constant.) Adiabatic expansion or contraction of the unit volume now extracts further work, until the radiation temperature is brought to equilibrium with the ambient temperature T_e . This amount of work is easily computed to be $aT_{\star}^4 + (1/3)aT_e^4 - (4/3)aT_{\star}^3T_e$. The total useful work is thus

$$R = E - ST_e + \frac{1}{3}aT_e^4 = G(T_e) \quad (1)$$

where $G(T_e)$ is the Gibbs free energy⁴ of the original unit volume evaluated relative to the ambient temperature T_e and its external radiation pressure $(1/3)aT_e^4$.

We must now compute S and E for the examples of interest: If $N dv d\Omega$ is defined to be the volume density of photons in a frequency interval dv and solid angle $d\Omega$, then (taking units with $c = 1$),

$$E = \int N h \nu dv d\Omega$$

$$S = \iint \left[2\nu^2 \ln \left(1 + \frac{N}{2\nu^2} \right) + N \ln \left(1 + \frac{2\nu^2}{N} \right) \right] dv d\Omega \quad (2)$$

The integration extends over all angles, and from 0 to ∞ in ν . Equation (2) goes back to Planck⁵ and others⁶.

Equations (2) and (1) could now be evaluated numerically using the empirically known radiation field near the Earth. A useful approximation, however, is to idealise the field as the sum of various, possibly diluted, black-body contributions. Such a flux with dilution factor ϵ has $N = 2\epsilon\nu^2/(\exp(h\nu/kT) - 1)$, and if it is from solid angle ω , then the integrals (2) can be done by computer and expressed as an accurate numerical approximation:

$$E = aT^4(\omega/4\pi)\epsilon \quad (3)$$

$$S = (4/3)aT^3(\omega/4\pi)\epsilon(0.9652 - 0.2777 \ln \epsilon - (0.0348 + f(\epsilon))\epsilon)$$

where $f(\epsilon)$ is a smooth function which can be neglected for $\epsilon < 0.01$; $f(1) = 0$, $f(0.1) = 0.0114$, $f(0.01) = 0.012$.

Consider now direct sunlight: The solar black-body contribution is undiluted at temperature T_{\odot} and occupies a fractional solid angle $\omega/4\pi = 5.4 \times 10^{-6} \equiv \delta$ (the size of the Sun in the sky). The remaining solid angle is approximately a black-body of temperature T_e , also undiluted. Combining equations (2) and (3) then gives

$$R = \delta a T_{\odot}^4 \left[1 - \frac{4}{3} \frac{T_e}{T_{\odot}} + \frac{1}{3} \frac{T_e^4}{T_{\odot}^4} \right] \quad (4)$$

The term in square brackets is seen to be the optimal efficiency, and it has a numerical value 0.93 for $T_{\odot} = 5,800$ K, $T_e = 300$ K. Next consider diffuse sunlight of intensity identical to the above. Here we set $\omega/4\pi = 1$ for both solar and ambient components, but set ϵ , the dilution factor, to δ for the solar contribution, and $\epsilon = 1 - \delta$ for the ambient contribution. Now

equations (2) and (3) combine to give (for $1 \gg \delta \gg \exp[-T_{\odot}/T_e]$)

$$R = \delta a T_{\odot}^4 \left[1 - \frac{4}{3} \left(0.9652 - 0.2777 \ln \delta \right) \frac{T_e}{T_{\odot}} + O \left(\frac{T_e^4}{T_{\odot}^4} \right) \right] \quad (5)$$

The numerical value of the bracketed efficiency coefficient is here 0.70. Diffuse sunlight, then (or direct sunlight with a conversion scheme which does not make use of its directivity), allows about 25% less conversion of energy. This is not simply the geometrical effect of increased flux on to a normal surface (the radiation being sampled on a volume basis), nor is it the effect of a lower total flux which is the general concomitant of diffuse radiation (since we have equalised the intensities in the above calculation); rather it is a consequence of the fundamentally greater entropy in the diffuse radiation, hence its smaller free energy.

To decide whether this is an important efficiency difference, we can note that even on a cloudless day with the Sun directly overhead, of order 20% of the total solar flux is diffuse, and an average temperate cloud cover raises this fraction towards unity with 60% typical⁷. One concludes that any scheme for using diffuse sunlight at near-maximum efficiency (and direct sunlight therefore at an automatic sacrifice of only $\sim 25\%$), should dominate a scheme which optimises for direct flux (and, for example, with focusing mirrors, sacrifices diffuse radiation almost completely).

In a sense, we were anticipated in this conclusion by the evolutionary experience of natural plants. The advantage of a differently "designed" photosynthesis at the photomolecular level, one which might use the directionality of the solar radiation on angular scale $\sim 0.5^\circ$, does not seem to have been sufficient to have driven terrestrial evolution in this direction. One is left to speculate about whether exobiological evolution under different conditions might be able to find a different chemistry for photosynthesis, one which uses the free energy of directionality in addition to (or instead of!) the free energy of spectral temperature.

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Reduction of visibility by sulphates in photochemical smog

THE relationship between pollutant emissions and the optical haze characteristic of photochemical smog has proved difficult to unravel^{1,2}. It is clear that material produced by reaction in the atmosphere is responsible for much of the deterioration in the optical environment, since the ambient aerosol scatters much more light at a given mass concentration than do the primary aerosols emitted by known sources^{3,4}. Unfortunately, measurement of the scattering contributed by an individual product species is complicated by the fact that most of this secondary material is deposited on existing particles and

cannot be isolated for direct observation of its *in situ* optical properties. In this note I present statistical evidence from measurements in Los Angeles that secondary sulphate compounds scatter visible light more efficiently than do other chemical fractions of the ambient aerosol. My results indicate that sulphur emissions can strongly influence atmospheric visibility even in photochemical smog of the Los Angeles variety, where secondary organics and nitrates contribute most of the pollutant aerosol mass.

Visibility in a polluted atmosphere is determined primarily by the total scattering coefficient (b_{scat}) of the ambient aerosol⁶. As part of the California Aerosol Characterisation Study (ACHEX)⁷, the scattering coefficient, mass concentration, and chemical composition of the Los Angeles aerosol were monitored during selected photochemical smog episodes in the summer of 1973. Of particular interest are data from 28 two-hour sampling periods of low ($\leq 60\%$) relative humidity, when scattering from water absorbed by hygroscopic constituents of the aerosol was minimal^{8,9}. The scattering coefficient averaged $6.4 \times 10^{-4} \text{ m}^{-1}$ over this sample, and showed significant correlation with aerosol mass concentration and sulphate concentration. Ninety per cent of the observed variance in the scattering coefficient is accounted for by the following least-squares fit (NSA: non-sulphate aerosol):

$$b_{\text{scat}} = 7.6 \text{ m}^2 \text{ g}^{-1} [\text{SO}_4^{2-}] + 2.4 \text{ m}^2 \text{ g}^{-1} [\text{NSA}] \pm 1.0 \times 10^{-4} \text{ m}^{-1}$$

In this expression, $[\text{NSA}] = [\text{aerosol}] - 1.3 [\text{SO}_4^{2-}]$ is the calculated concentration of non-sulphate aerosol, the factor 1.3 representing the mass ratio of sulphate compound to sulphate ion.

The value 1.3 used above for the compound/ion (C/I) ratio was chosen as representative of the likely atmospheric mix of $(\text{NH}_4)_2\text{SO}_4$ (C/I = 1.38), $(\text{NH}_4)\text{HSO}_4$ (C/I = 1.20), Na_2SO_4 (C/I = 1.48), NaHSO_4 (C/I = 1.25), and H_2SO_4 (C/I = 1.02), based on the relative concentrations of sulphate, nitrate, ammonium, and sodium found in the aerosol¹⁰. The compound/ion ratio can also be estimated by purely statistical means, and this provides a way to test the experimental data for the possible influence of "hidden" variables statistically associated with sulphate. Multiple linear regression of aerosol mass concentration on $[\text{SO}_4^{2-}]$, $[\text{NO}_3^-]$, and $[\text{C}]$ over all relative humidities gives a value of 1.2 ± 0.2 for the $[\text{SO}_4^{2-}]$ coefficient, which is consistent with the compound/ion ratios of the probable aerosol sulphates identified above. (The $[\text{SO}_4^{2-}]$ coefficient in the corresponding regression of b_{scat} on $[\text{SO}_4^{2-}]$, $[\text{NO}_3^-]$, and $[\text{C}]$ over all relative humidities is $9.8 \text{ m}^2 \text{ g}^{-1}$.) The accuracy of the statistical estimate for the contribution of sulphates to aerosol mass lends credibility to my statistical estimate for the contribution of sulphates to light scattering.

The empirical b_{scat} -aerosol relationship presented here indicates that sulphate compounds scatter much more efficiently at low humidities than the remainder of the aerosol does. During the sampling intervals for which the relationship was derived, sulphate compounds apparently contributed almost a third of the average scattering coefficient, although they contributed only $30\text{--}35 \mu\text{g m}^{-3}$ ($[\text{SO}_4^{2-}] = 25 \mu\text{g m}^{-3}$) towards the average mass concentration of $220 \mu\text{g m}^{-3}$. The relationship was fitted to a limited set of data from a limited geographical area, and should be applied to other situations only with considerable caution. It is worth noting, however, that the reciprocal of the $[\text{SO}_4^{2-}]$ coefficient falls within the range of $[\text{SO}_4^{2-}]/b_{\text{scat}}$ ratios reported by Waggoner *et al.*¹¹ for aerosols dominated by sulphates. This raises the possibility that the optical activity of the sulphate compounds results from basic physical and chemical mechanisms which govern their distribution in the aerosol with respect to particle size, as suggested in a more general context by Friedlander¹². Further measurements will be necessary before this hypothesis can be confirmed or disproved.

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Exsolution in 'stoichiometric' mullite

MULLITE, first identified only fifty years ago, was quickly recognised to be an important industrial mineral. Its chemical composition was determined initially as $3\text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2$ on the basis of two chemical analyses^{1,2} and the observation that when a synthetic mixture of 3:2 composition was fired to temperatures just below the liquidus, no glass or corundum was observed¹. Although more aluminium-rich mullites were found subsequently³, the 3:2 or "stoichiometric"⁴ composition remains the generally accepted one.

In the course of a study of solid solution in mullite a number of occurrences of rocks with coexisting sillimanite and mullite were found⁵. The most obvious way that this could come about would be if reactions (1) or (2) had not gone to completion:



Reaction (1), a common synthetic mullitisation process, takes place at about $1,400^\circ\text{C}$ at 1 atmosphere while thermodynamic calculations^{6,7} indicate that mullite should form according to reaction (2) at about 900°C . Two specimens of

Fig. 1 Mullite with exsolved sillimanite, specimen Al 37y, Thorncliffe, Transvaal, South Africa. *a*, Overfocused optical micrograph of a grain mounted in oil at 45° position under crossed polars. *b*, Electron micrograph showing lamellae of sillimanite (light) in a mullite host.

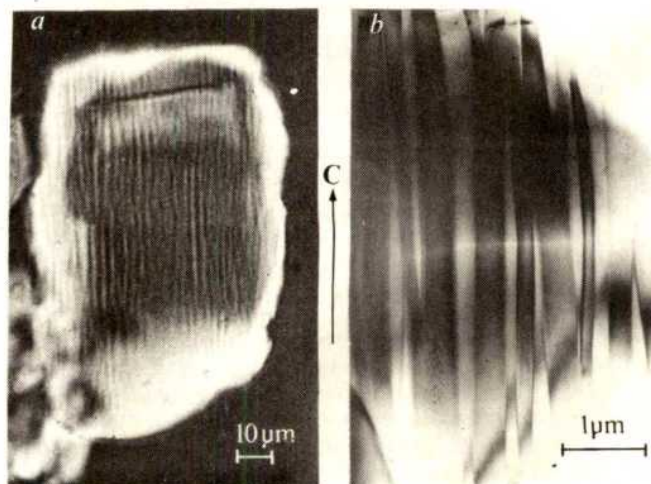


Table 1 Chemical compositions and cell dimensions of the sillimanite and mullite phases

	Electron probe		EMMA-4	
	"Mullite"	Sillimanite	Mullite host	Sillimanite
Al ₂ O ₃	69.7	63.0	74.1	63.7
SiO ₂	28.9	36.1	24.4	35.8
TiO ₂	0.8	0.07	—	—
Fe ₂ O ₃	0.3	0.3	—	—
Cr ₂ O ₃	0.3	0.2	—	—
Total	100.0	99.7	98.5	99.5
Mol % Al ₂ O ₃ + TiO ₂ + Fe ₂ O ₃ + Cr ₂ O ₃	59.0(5)	50.9(5)	64.5(2.0)	51.4(2.0)
Cell dimensions		Mullite	Sillimanite	
<i>a</i> (Å)		7.5482(7)	7.4861(9)	
<i>b</i> (Å)		7.6824(15)	7.6827(21)	
<i>c</i> (Å)		2.8876(3)	2.8879(4)	
<i>V</i> (Å ³)		167.45(3)	166.09(4)	

Experimental details for the electron probe and powder X-ray work as in ref. 5. EMMA-4 instrumental conditions are those described by Cliff and Lorimer¹³. Al/Si ratios were recalculated as oxides to 100%, allowing for estimated trace element contents. The results quoted are the average of 10 analyses. Figures in parentheses represent the standard deviation in terms of least units cited for the value to their immediate left.

corundum-sillimanite-mullite xenoliths from different localities within the Critical Zone of the Bushveld Intrusion⁸ were sufficiently coarse grained to afford detailed examination under the polarising microscope. The absence of a silica phase either within mullite grains or at grain boundaries suggests that reaction (2) was the mullite-forming process. Indeed, in specimen Al 37y, sillimanite is found preferentially in contact with (?)relict corundum grains.

The ratio of sillimanite to mullite, estimated optically, in specimens Al 37y and JW II 5 was very much less than that determined by X-ray powder diffractometry⁵. Coupled with the fact that sillimanite, not mullite, is found in the outermost zone of the Thorncliffe xenolith where it reacted *in situ* with an anorthositic host, there seemed a strong possibility that earlier formed mullite in the core of the xenolith may have exsolved sillimanite after settling near the floor of the magma chamber. In thin section individual grains have a distinctly mottled appearance under crossed polars and, when mounted in oil, almost all show evidence of microstructure parallel to the *c* axis of the crystals on a scale of about 0.1 µm both under crossed polars (Fig. 1a) and in plane polarised light. This implies that a second phase with a slightly different refractive index is present; indeed the overall texture is strongly reminiscent of exsolved orthoamphiboles⁹ and materials that have undergone spinodal decomposition but on a very much coarser scale¹⁰.

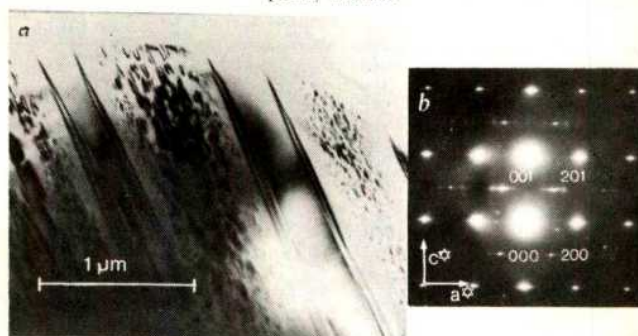
An electron microscope study (AEI EM6G) of ion-thinned crystals from both specimens revealed sinuous lamellae up to 0.2 µm wide and attaining lengths of several tens of microns (Fig. 1b). Diffraction patterns from the lamellae showed the simple 2*c* superlattice reflections characteristic of sillimanite. The host displayed intensity maxima at irrational values of *h*, *k* and *l*, typical of the antiphase domain structure adopted by mullite¹¹. The plane of contact is (100), that of the greatest mismatch between the two unit cells (about 1% from Table 1). Within the mullite phase are tiny platelets (0.1 × 0.012 µm) which become smaller and finally disappear near sillimanite lamellae, leaving a precipitate-free zone 0.05–0.15 µm wide (Fig. 2a). The *a**–*c** diffraction pattern (Fig. 2b) taken from an area 0.5 µm in diameter in the mullite host midway between two sillimanite lamellae shows that the platelets also have the sillimanite structure and are in precisely the same crystallographic orientation as the mullite.

One must conclude that sillimanite exsolves on (100) of mullite during very slow cooling. The wide lamellae are likely to have formed at temperatures as high as 1,100 °C but diffusion of Al (and Si) was sufficiently sluggish for a concentration gradient to be set up in the mullite host, resulting eventually in the exsolution of a second generation of sillimanite. Augite exsolution from orthopyroxene is an almost perfect analogy¹² except that in the case of mullite the tiny platelets within the host seem to be those of the equilibrium phase and are there-

fore not Guinier–Preston zones. A certain amount of disorder does exist, however, in the sillimanite as evidenced by streaking in the diffraction pattern parallel to *a**.

The bulk composition, that of the initial mullite, was found by electron probe microanalysis (beam diameter 2 µm) to be 59.0 mol % Al₂O₃ (+ TiO₂ + Fe₂O₃), only slightly off the 3:2 stoichiometry (Table 1). The quoted sillimanite analysis is that from a few crystals of apparently homogeneous sillimanite in the Thorncliffe specimen and is assumed to be similar to that of the coarsely exsolved lamellae. Two approaches towards finding the compositions of the exsolved phases were adopted. Use of the relationship between the *a* cell dimension and Al content in synthetic and natural mullites with very different thermal histories (W.E.C., unpublished) gave the chemical composition of the mullite lamellae as 61.3 ± 0.3 mol % Al₂O₃. The powder diffraction peaks of sillimanite were slightly broader than those of mullite, resulting either from the disorder mentioned previously or from a small compositional difference between the two scales of lamellae. The *a* parameter, however, was lower than those in sillimanites with similar minor element contents from metamorphic rocks formed at similar pressures while the cell volume was typical of sillimanites with a small amount of excess Al. EMMA-4 analyses (beam diameter 0.1 µm) using *k* values¹³ determined on the Brandywine Springs sillimanite and an analysed mullite, 76398 (ref. 14), are given in Table 1. Thin, precipitate-free mullite lamellae were chosen and for all analyses, great care had to be exercised in determining background levels. EMMA results, in spite of their high estimated errors, confirm the presence of excess aluminium

Fig. 2 Nature of the precipitates within the mullite host. *a*, Electron micrograph showing the second generation of exsolved sillimanite. Contrast fringes at the sillimanite-mullite interfaces arise from inclined planar defects, observed when the beam is not quite perpendicular to (010). *b*, *a**–*c** electron-diffraction pattern from the mullite host, indexed on the *c* = 2.9 Å cell. The sharp superlattice reflections characterise sillimanite; the diffuse pairs, mullite.



in the sillimanite and provide a very much more reasonable value for the mullite composition considering the volume relationships shown in Fig. 1b. The cell dimensions, in any case, are likely to be suspect owing to the possibility of coherent or semi-coherent exsolution, as in perthites¹⁵. The actual mechanism of exsolution is most likely to have been by nucleation and growth, in spite of the even distribution of precisely oriented sillimanite lamellae which one might ascribe to a coarsened spinodal. The solute profile inferred for the mullite host is the reverse of that which would arise by spinodal decomposition.

The observation that mullite has exsolved to give a non-stoichiometric sillimanite and another mullite with about 64 mol % Al_2O_3 argues very strongly against the presence of any 3:2 "ordered"¹⁶ compound at high temperature. Slowness of diffusion of Al and Si was the main constraint on the decomposition reaction and instead of nucleating the thermodynamically stable phase, corundum, the host phase preserved the topology of an initially crystallised mullite. The Bushveld specimens represent the closest approach to equilibrium for reverse reaction (2) that is ever likely to be found: makers of mullite refractories need have little cause for concern.

This paper has benefitted greatly from the technical skill and advice of Mr G. Cliff and from discussions with Drs G. W. Lorimer, P. E. Champness and J. D. C. McConnell. Mr E. A. Viljoen kindly provided the Bushveld specimens.

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Transfer of chlorinated biphenyls to Antarctica

PREVIOUS determinations of synthetic organic compounds in antarctic snow¹ and in tissues of species inhabiting the antarctic marine environment^{2–5} have shown the presence of DDT compounds in almost all samples examined, but the apparent absence of polychlorinated biphenyls (PCB). PCB, however, are ubiquitous marine pollutants north of

Table 1 DDT and PCB in Antarctic snow from Doumer Island, January–February 1975

Depth (m)	Concentration ($10^{-15} \text{ g g}^{-1}$)			$\Sigma \text{DDT/PCB}$
	<i>p,p'</i> -DDT	<i>p,p'</i> -DDE	PCB	
0–0.5	500	130	300	2.1
0–0.5	550	58	170	3.6
0–0.5	440	54	30	16
0.5–1.0	450	67	57	9.1
1.0–1.5	1,300	62	220	6.2
1.3–1.6	2,700	220	1,200	2.4
3.5–4.0	4,000	270	400	11
4.0–4.5	3,000	240	680	4.8
4.5–5.0	2,800	170	430	6.9
5.0–5.5	3,400	380	810	4.7
5.5–6.0	2,100	210	280	13

the Antarctic Convergence^{2,5–8} and were detected in all eggs and tissues of seabirds examined from the subantarctic Auckland Islands, centred at 166°05'E, 50°40'S. These data have been used to support the hypothesis that the atmosphere is not the primary medium of transport of this class of pollutants to remote environments such as Antarctica¹. In the present paper we report the detection of PCB in the eggs of resident penguin species, by removal of unknown compounds previously interfering in the analysis, and in antarctic snow, by application of *in situ* extraction in the field of large volumes of melted snow. Equivalent levels and ratios of these pollutants in species resident north and south of the Convergence indicate atmospheric, rather than oceanic, transport to Antarctica.

In January 1975, a field camp was established on Doumer Island (63°35.5'W, 64°51.3'S) on the Antarctic Peninsula. Ninety-nine litre samples of melted snow were extracted *in situ* by passage through a glass column (length 30 cm, internal diameter 3 cm) packed with polyurethane foam plugs (81% polyether glycol, 19% toluene di-isocyanate, density 0.036 g cm⁻³, United Foam Company, Los Angeles). The packed columns and all equipment were cleaned in the laboratory with successive washes of acetone until background levels of DDT and PCB compounds were less than $10^{-14} \text{ g g}^{-1}$ in a 100-l sample; all equipment was wrapped tightly in clean aluminium foil until used in the field. Controls included columns transported to Antarctica, which were not opened, and which were returned to the laboratory for analysis. Samples obtained at depths greater than 2 m were obtained by excavating in the wall of a small crevasse. Results are presented in Table 1; analytical techniques have been reported elsewhere⁵. The concentrations of total DDT, ranging from 0.5 to $4 \times 10^{-12} \text{ g g}^{-1}$, are in the same range but somewhat higher than those previously reported by Peel¹ from an area considerably further south (26°42'W; 75°31'S). Concentrations of PCB, principally pentachlorobiphenyls, ranged between 0.03 to $1.2 \times 10^{-12} \text{ g g}^{-1}$; the median $\Sigma \text{DDT} : \text{PCB}$ ratio was 6.2.

Elsewhere⁴, the hypothesis that the majority of the organochlorine pollutant residues in Antarctica have

Table 2 PCB and DDE concentrations in eggs of penguins from the Antarctic Peninsula

Species	Date	Locality	N	% H ₂ O	% lipid	<i>p,p'</i> -DDE†	PCB†
<i>P. adeliae</i>	Jan 1970	Anvers Island	14	Not determined	11.8 (7.0–14.6)	137 (65–330)	81 (40–180)
	Dec 1973	Anvers Island	15	74.4 (69–80)	8.2 (5.5–10.6)	204 (86–310)	73 (27–160)
	13 Jan 1975	Anvers Island	12	73.0 (70–78)	9.7 (5.6–13.0)	139 (72–200)	56 (27–93)
<i>P. antarctica</i>	11 Jan 1975	Gibbs Island*	10	75.1 (69–78)	10.8 (4.9–16.4)	172 (90–380)	43 (21–80)
	15 Jan 1975	Doumer Island	8	75.0 (73–79)	9.0 (6.5–10.8)	168 (110–310)	72 (27–140)

* Approximately 55.6°W, 61.5°S.

† Lipid basis, 10^{-9} g g^{-1} . Geometric means with the range.

derived from local human activities has been examined and rejected; ratios among the compounds detected are not consistent with those predicted. Potential local sources of PCB contamination on Doumer Island included the small Chilean base Yelcho, 2 km distant from the sampling site, which has been occupied intermittently over the past few years, and Palmer Station, the much larger American base to the west on Anvers Island (64°3'W, 64°46.3'S). Local contamination of PCB on Anvers Island was shown by analysing four samples of surface snow obtained on February 13–16, 1975, 0.5–1.5 km from Palmer Station. This snow had a high content of particulates, principally soot, and contained from 4 to $10 \times 10^{-12} \text{ g g}^{-1}$ of total PCB. We conclude that the PCB residues most probably derived from the frequent burning in the past of considerable quantities of waste materials at Palmer Station. A portion of the PCB residues detected on Doumer Island may therefore derive from local input.

Widespread distribution of PCB in the Antarctic was, however, demonstrated by the analysis of diverse members of the food web from separated localities. The results of the analysis of eggs of three species of penguins obtained between January, 1970, and January, 1975, are presented in Table 2. All extracts contained compounds, presumably of natural origin, which produced peaks on electron capture gas chromatograms which interfered with the detection of polychlorinated biphenyls. These compounds were destroyed, however, by rigorous saponification; a saponification side column was therefore used in the analysis of these samples⁹.

No significant differences in concentrations of *p,p'*-DDE or PCB were found among the three species obtained in 1975 (Wilcoxon 2-sample test, $P > 0.05$). PCB concentrations, however, were significantly lower ($P < 0.001$) in Adelie Penguin eggs obtained in 1975 than in those obtained in 1970. Median DDE:PCB ratio in the penguin eggs was 3.0: concentrations of *p,p'*-DDT in the eggs were usually a small fraction of the concentration of *p,p'*-DDE.

Other samples examined for the presence of PCB included the blubber of a leopard seal (*Hydrurga leptonyx*), which had been killed by killer whales (*Orcinus orca*) in the Gerlache Strait (62°10'W, 64°30'S) on October 30, 1975. Concentrations in the fat of *p,p'*-DDE, *p,p'*-DDT and PCB (principally pentachlorobiphenyls) were 48, 33 and $43 \times 10^{-9} \text{ g g}^{-1}$, respectively. These levels are equivalent to those in the penguins, suggesting that this animal had not been feeding on penguins, but rather on a common food source such as krill. A sample of krill (*Euphausia* sp.), obtained at a depth of 250 m on October 31, 1975, at 60°27'W, 62°39'S with an Isaac Kidd midwater trawl, contained 14, 19 and $3 \times 10^{-9} \text{ g g}^{-1}$ respectively of *p,p'*-DDE, *p,p'*-DDT and PCB (pentachlorobiphenyls) on an extractable lipid basis.

The concentrations of *p,p'*-DDE and PCB in penguin eggs from the Antarctic Peninsula are equivalent to levels recorded in four eggs of the rockhopper penguins (*Eudyptes crestatus*) from the Auckland Islands in 1972–73. Moreover, DDE:PCB ratios in eggs of the Auckland island shag (*Phalacrocorax carunculatus*) ranged between 3.2 and 4.0; the ratio in the fat of a Hooker's sea lion (*Otaria hookeri*) was 5.1. Comparable DDE:PCB ratios were recorded in species from other areas of the New Zealand subantarctic and coastal New Zealand⁹. These ratios, typical also of the Antarctic, reflect the relative usage and environmental input of DDT and PCB compounds into the Southern Hemisphere¹⁰; in the Northern Hemisphere PCB compounds usually predominate over the DDT group in marine samples^{9–8}. Comparable levels of organochlorine contamination north and south of the Antarctic Convergence, which separates sharply defined and distinct water masses, indicate that the atmosphere is the dominant pathway of transport of both PCB and DDT compounds to the Antarctic.

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²⁴¹Americium in Mediterranean surface waters

LITTLE information is available to date on the occurrence of ²⁴¹americium in sea water, except for data obtained in some special areas such as in the vicinity of a nuclear reprocessing plant (Windscale, UK)¹ or in areas of close-in fallout from nuclear explosion tests (Bikini and Eniwetok Atolls, Pacific)². So far, only a few data of ²⁴¹Am obtained from one station in the North Atlantic have been published by Livingston *et al.*³. This is mainly due to analytical difficulties involved in determining extremely low concentrations of ²⁴¹Am in open ocean waters. However, since the inventory of ²⁴¹Am in radioactive wastes from nuclear fuel reprocessing is expected to exceed that of ²³⁹Pu plus ²⁴⁰Pu in the near future, it is important to start obtaining baseline data of ²⁴¹Am in oceanic waters now, in order to follow the future trend of concentration changes, if any, of this isotope.

We have determined the concentration of ²⁴¹Am in surface waters of the Mediterranean Sea, collected from several stations covered during the 1975 cruises. The detailed analytical procedures employed were described by Ballestra *et al.*⁴. Briefly, ²⁴¹Am together with plutonium isotopes were coprecipitated with mixed hydroxides and carbonates of calcium and magnesium, which are naturally present in seawater, from approximately 200 l of unfiltered seawater; a second coprecipitation was carried out by scavenging with $\approx 50 \text{ mg Fe}^{3+}$. ²⁴¹Am was purified from plutonium isotopes as well as other interfering α -emitters such as U, Po, and Th, by cation and anion exchange and solvent extraction with HDEHP (di-2-ethylhexyl phosphoric acid-heptane); finally ²⁴¹Am was electrodeposited on to a stainless steel disk and α -spectrometrically determined by using a silicon surface barrier detector. The chemical yield of ²⁴¹Am for the separation procedure, determined by the initial addition of known amounts of ²⁴³Am, was 40–70%.

The results of these measurements are given in Table 1. In this table the results for ²³⁸Pu and ²³⁹+²⁴⁰Pu are also presented. Considering the errors involved in the measurements, the data in Table 1 show rather homogeneous distributions of these isotopes in the surface layer of the Mediterranean. On the basis of these data average concentrations of ²⁴¹Am, ²³⁸Pu and ²³⁹+²⁴⁰Pu in surface water of the Mediterranean are calculated

Table 1 Results of measurements of transuranic elements in Mediterranean surface waters

Position	Date of collection	Chlorinity (‰)	$^{239+240}\text{Pu}^*$ (fCi l $^{-1}$)	$^{238}\text{Pu}^*$ (fCi l $^{-1}$)	$^{241}\text{Am}^*$ (fCi l $^{-1}$)
43°39'N, 12°00'E	2 July '75	21.26	1.35 ± 0.06	0.06 ± 0.01	0.06 ± 0.01
43°11'N, 06°32'E	14 Sept '75	—	0.91 ± 0.07†	0.10 ± 0.03†	0.03 ± 0.01†
38°30'N, 06°30'E	17 Sept '75	20.56	0.77 ± 0.06	0.07 ± 0.02	0.08 ± 0.02
37°30'N, 06°30'E	18 Sept '75	20.78	0.93 ± 0.07	0.06 ± 0.01	0.09 ± 0.02
38°40'N, 12°00'E	20 Sept '75	20.12	1.04 ± 0.06	0.05 ± 0.01	0.05 ± 0.02
40°40'N, 11°40'E	20 Sept '75	21.18	1.09 ± 0.09	0.05 ± 0.01	0.07 ± 0.02
41°20'N, 11°30'E	21 Sept '75	21.11	0.95 ± 0.09	0.06 ± 0.02	0.05 ± 0.01
42°47'N, 09°25'E	21 Sept '75	21.13	1.1 ± 0.1	0.06 ± 0.02	0.03 ± 0.01
43°55'N, 09°00'E	22 Sept '75	21.17	1.10 ± 0.06	0.10 ± 0.02	0.05 ± 0.01

* Errors indicated are 1σ propagated errors.

† The water sample was filtered through HA Millipore (0.45 μm pore size).

to be 0.057 ± 0.007 , 0.068 ± 0.007 and 1.03 ± 0.05 fCi l $^{-1}$ respectively.

These average values give the following activity ratio

$$^{241}\text{Am}/^{239+240}\text{Pu} = 0.055 \pm 0.007$$

As to the $^{241}\text{Am}/^{239+240}\text{Pu}$ ratio only one value 0.033, obtained for the Lake Michigan water, has been reported⁵. The similar ratio for North Atlantic surface water calculated on the basis of the data presented by Livingston *et al.*³ is 0.2 ± 0.1 . Our ratio is in reasonable agreement with that given for the Lake Michigan water. It is believed to be representative at least for surface waters of the Mediterranean and possibly also for surface layers of oceans in the temperate zone of the Northern Hemisphere, since fallout delivery ratio as well as mechanisms of removal from the upper mixed layer of these isotopes are not likely to be substantially different among different oceanic areas in this zone.

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Phosphate adsorption on goethite (α-FeOOH)

SYNTHETIC goethite has been extensively studied as a model adsorbent in the search for a precise description of the chemical reactions involved in phosphate adsorption by soil minerals. Infrared studies^{1,2} have now firmly established that phosphate on goethite and other iron oxides results in a bridging binuclear complex of the type Fe-O-P-O-Fe. The infrared studies were, however, carried out on dried samples of phosphated goethite. We now report infrared studies on wet goethite films which

confirm that the binuclear complex exists in the presence of water.

Furthermore, infrared spectra of phosphate adsorbed on goethite show differences according to the suspension pH at which the sample was prepared^{1,3}. These probably arise from different states of protonation of the adsorbed phosphate, but the results have been difficult to interpret. The possibility of different states of protonation of adsorbed phosphate is suggested also by potentiometric titration measurements⁴⁻⁷ of the surface charges of phosphated and non-phosphated goethite and other minerals, but this method cannot distinguish between phosphate groups and other surface sites as locations for hydrogen ions. It seemed desirable to combine the two methods, using comparable samples, and by doing so we are now able to report an improved interpretation of the spectral changes caused by changes in the state of protonation of adsorbed phosphate on goethite.

The synthetic goethite was prepared by the usual methods⁸ (OH/Fe 1.5, 50-h ageing). The phosphate adsorption capacity of 197 μmol g $^{-1}$ at pH 3.3 indicated⁹ a surface area of ~ 80 m² g $^{-1}$. This corresponds to ~ 400 μmol singly co-ordinated OH $^{-}$ or H₂O (A-type²)/g of goethite.

Phosphated goethite suspensions were prepared for infrared spectroscopy using almost completely adsorbed amounts of phosphate (see Table 1). The samples were drained to water contents of ~ 1.0 g of H₂O/g of goethite, on AgCl plates. The thickness of the water film around each goethite crystal was estimated to be 10–15 nm at this water content.

The infrared spectra of the goethite suspensions phosphated with H₃PO₄ (pH 3.6 or 5.1) showed two phosphate bands near 1,100 and 1,000 cm $^{-1}$ (Table 1). These bands arise from the P=O and P-O-(Fe) stretching vibrations of phosphate adsorbed in a binuclear bridging complex (FeO)₂POOH (refs 2, 3). Drying caused the P=O band to shift to higher frequencies as the influence of hydrogen bonded water dwindled. On treating the evacuated complexes with D₂O and re-evacuating, the phosphate bands shifted to different frequencies, indicating that the phosphate group was protonated.

When NaH₂PO₄ (pH 8.1) or Na₂HPO₄ (pH 9.7) was added to the goethite suspensions, phosphate bands were observed near 1,080 and 1,040 cm $^{-1}$. Drying caused the 1,080 cm $^{-1}$ band to shift to a higher frequency (air dry, 1,105 cm $^{-1}$; evacuated, 1,192 cm $^{-1}$) while the 1,040-cm $^{-1}$ band shifted to a lower frequency (1,000 cm $^{-1}$) characteristic of the binuclear complex. The 1,080 and 1,040 cm $^{-1}$ bands returned immediately on rewetting the goethite films. Since it is unlikely that rearrangement to a monodentate species could occur so readily, the most

Table 1 Absorption bands (ν(cm $^{-1}$)) of phosphated goethite

Phosphate added (μmol g $^{-1}$)	Suspension pH	In suspension			Evacuated		D ₂ O, evacuated	
		P=O	PO ₂ $^{-}$	P-O-(Fe)	P=O	P-O-(Fe)	P=O	P-O-(Fe)
200 H ₃ PO ₄	3.6	1,115	—	1,000	1,192	996	1,185	1,013, 991
100 H ₃ PO ₄	5.1	1,100	—	1,000	1,192	1,000, 986	1,183	1,000
100 NaH ₂ PO ₄	8.1	—	1,086	1,042	1,192	1,000	1,183	1,000
50 Na ₂ HPO ₄	9.7	—	1,070	1,040	1,192	1,000, 986	1,183	1,000

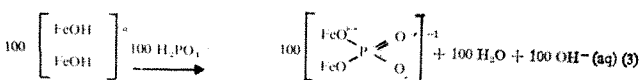
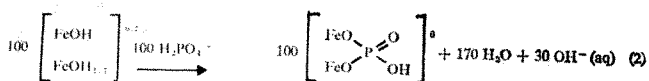
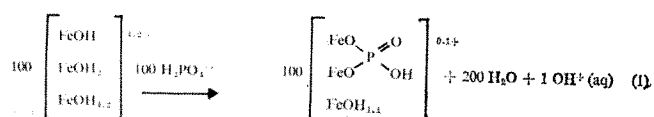
plausible explanation for the 1,040- and 1,080-cm⁻¹ bands is that the phosphate is in an ionised binuclear form (FeO)₂POO⁻Na⁺.

The phosphate bands for evacuated goethite from high pH suspensions are similar to the phosphate bands for evacuated goethite from low pH suspensions (Table 1), and the bands show similar shifts after exchange with D₂O. These results show that the (FeO)₂POONa complex becomes protonated when evacuated, probably by Na⁺ exchanging with H⁺ from Lewis acid sites on the (010) faces².

The potentiometric titration curves of goethite suspensions in NaCl solutions, with and without phosphate, are shown in Fig. 1. The phosphate addition was 100 µmol NaH₂PO₄ per g goethite, which was virtually completely adsorbed, and thus the results do not require corrections for the titration of non-adsorbed phosphate.

Surface-charge data can be obtained by choosing an origin which is the zero point of charge, located by the common point of intersection of the titration curves⁴, at pH 8.1 for non-phosphated goethite, and at pH 5.1 for phosphated goethite. A similar shift in the zero point of charge was observed for haematite, changing from pH 8.9 (non-phosphated) to pH 5.3 (phosphated)¹⁰. Other anions have similar effects⁴.

Surface-charge data obtained from the potentiometric titration curves in 0.01 M NaCl were used to construct equations (1), (2) and (3) which refer to pH 3.6, 5.1 and 8.1 respectively (selected to correspond with the spectroscopic sample preparation conditions). The equations are written in numbers of µmol, to facilitate comparison with experimental measurements.



The OH₂, OH, OH_{1.2} and so on, represent singly coordinated species² which can have an average state of protonation ranging between 1.0 and 2.0 depending on pH and ionic strength. Other species, which are bridging in the coordination shells of the iron atoms on the goethite surfaces, are not shown. The coordination (bridging binuclear) and the state of protonation of the adsorbed phosphate shown agree with the results from infrared spectroscopy.

The equations (1)–(3) predict the release of 10 µmol H⁺ per g goethite, 30 µmol OH⁻ per g goethite, and 100 µmol OH⁻ per g goethite at the selected pH values, 3.6, 5.1 and 8.1 respectively. To test these predictions goethite suspensions and phosphate (100 µmol g⁻¹) solutions at these pH values were flushed with nitrogen, mixed, and allowed to equilibrate under a nitrogen atmosphere. They were then titrated to the original pH values and the results showed that 16 µmol H⁺, 23 µmol OH⁻, and 80 µmol OH⁻ respectively were released during the adsorption reaction, in fair agreement with the predicted values. The consistency between potentiometric titration data and the findings from infrared spectroscopy support our conclusion that adsorbed phosphate on goethite is mostly protonated (HPO₄²⁻) at pH 5, and mostly deprotonated (PO₄³⁻) at pH 8.

Similar results were obtained from titrations carried out without nitrogen flushing, but the interpretation is more difficult because carbonate ions are adsorbed on goethite in

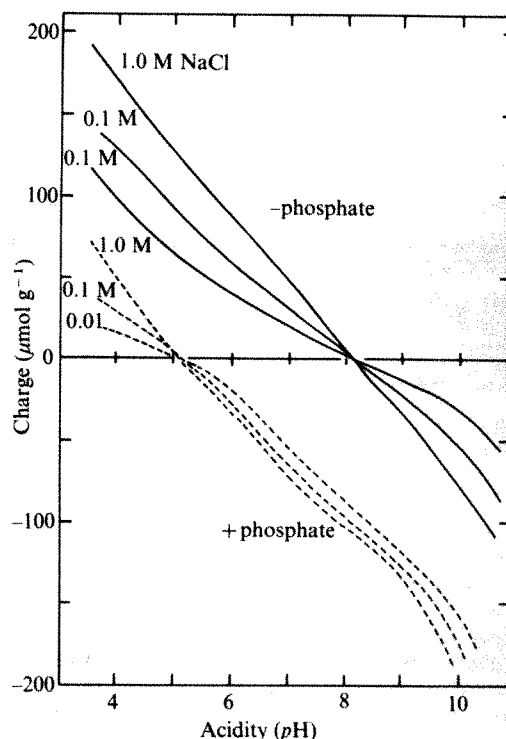


Fig. 1 Surface charges of goethite (—) and phosphated goethite (---), from potentiometric titrations of suspensions in various NaCl concentrations. Phosphated goethite was prepared by adding 100 µmol NaH₂PO₄ per g goethite.

these conditions¹¹. The apparent zero points of charge of goethite and phosphated goethite were pH 8.9 and 5.1 respectively, when nitrogen flushing was not used.

The equations (1)–(3) do not represent different types of adsorption reaction or different types of sites. In accordance with a proposed general model for anion adsorption on oxide surfaces¹², equations (1)–(3) represent average states of protonation of surface ligands. For example, at some pH value between 5 and 8 we may expect to find equal numbers of protonated and deprotonated adsorbed phosphate.

These results extend the work reported by others^{6–7} in two important respects. First, we have used infrared spectroscopy to examine the coordination and state of protonation of adsorbed phosphate, in wet pastes and *in vacuo*. Second, we have found that equations based on surface-charge measurements by potentiometric titration, and on infrared spectroscopy results, give reasonable agreement between predicted and observed H⁺ release or OH⁻ release during phosphate adsorption on goethite.

Recent work suggests that binuclear phosphate complexes occur also on gibbsite surfaces^{13,14}.

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Possible interstadial and interglacial pollen floras from Teindland, Scotland

THIS paper reports new investigations carried out on the Teindland soil profile first described by E. A. FitzPatrick¹. The section (grid ref. NJ 3297 8570) lies at an altitude of about 101 m OD in a disused quarry within Teindland Forest, 5 km south-west of Fochabers in Grampian Region, Scotland. The present study presents evidence which suggests that a fossil microflora contained within the soil can be assigned to two distinct phases of geological time, the Middle Devensian interstadial and the Ipswichian stage. This is augmented by stratigraphic analyses which suggest that the site is of wider significance for the Quaternary chronology of the area than has been appreciated.

The stratigraphy of the Teindland profile as described by FitzPatrick may be summarised as follows. The section showed at the top a semi-podsol in 2–2.4 m of sandy till and outwash gravel which overlay a fossil iron podsol developed in fluvoglacial outwash. The upper part of the buried soil which was originally the organic and leached layers, had been subjected to solifluction processes, producing thin bands of black and grey material. A radiocarbon assay from the organic black bands rendered a date of 28,140±480 and –450 years b.p. (NPL-78). FitzPatrick interpreted the history of the section thus: (1) Glaciation (Riss? = Wolstonian?) followed by the deposition of fluviglacial deposits. (2) Pedogenesis in the fluviglacial deposits of (1) during an interglacial (= Ipswichian?) to produce a well developed iron podsol. (3) Periglacial conditions causing disturbance and solifluction of the surface layer. (4) A second period of glaciation (Würm? = Devensian?) during which the soil was buried after 28,140 b.p. beneath till and outwash. (5) Holocene (= Flandrian) pedogenesis in the upper part of the Devensian deposits.

Pollen analysis and limited stratigraphical investigation were carried out on the Teindland profile in order to test FitzPatrick's interpretation of the site's evolution, and to establish whether evidence could be found for environmental conditions in the largely unknown Middle Devensian and Ipswichian stages in Scotland.

The stratigraphy at the point of sampling is shown in Fig. 1. Samples for pollen analysis were treated by Erdtman's acetolysis and hydrofluoric acid digestion². Preparations were mounted in silicone oil. There was a general scarcity of pollen and the lowest count for which there is percentage representation in the diagram is 126 land pollen grains (320 cm). Three levels contained no pollen (230, 289 and 310 cm) and elsewhere only traces were found. To facilitate discussion, the pollen diagram (Fig. 2) is zoned biostratigraphically (Table 1)³, though it will be apparent that the presence of no more than traces of pollen at some levels makes boundary location somewhat arbitrary, and the zones are partly discussed out of strict numerical sequence.

As might be expected from a profile which has had a long and varied history, the contained pollen record is not simple. The surface sample (zone T-6) with its *Pinus*, *Calluna vulgaris* and Gramineae assemblage differs from the spectra in underlying zone T-5 which is presumed to

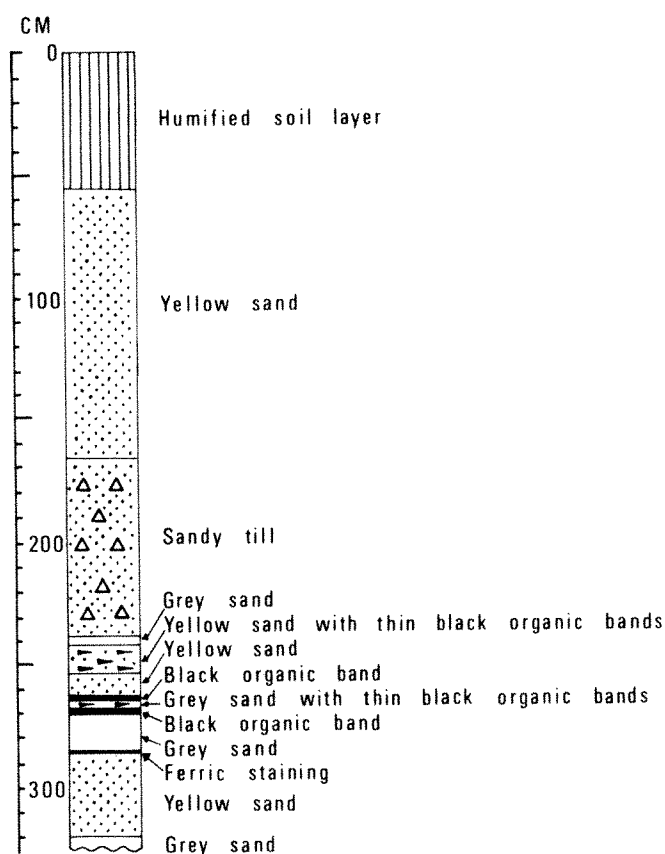


Fig. 1 Stratigraphy.

represent the heath-type vegetation cover prior to the planting of *Pinus sylvestris* woodland about forty years ago. The homogeneous nature of the spectra in zone T-5 is probably due to a process of microfaunal mixing of pollen and/or downflow of pollen through the soil profile^{4,5}. Zones T-5 and T-6 are also quite different from zone T-4, represented by a spectrum obtained from sand within the till (the only till sand sample to produce a large pollen count). This suggests that any downflow of microfossils through the upper soil over the last forty years, or through the till at any stage, has been negligible.

The *Alnus*, Gramineae, *Plantago lanceolata* pollen assemblage of zone T-4 is remarkably similar to the spectra of basal zone T-1. This is a feature of the utmost interest. These zones alone contain high frequencies of *Alnus* and *Plantago lanceolata* pollen. The location of zone T-1 below organic levels dated to the Middle Devensian (zone T-3), and the arboreal content of zone T-1 lead us to infer that these spectra represent a record of part of an interglacial landscape within north-east Scotland, chronologically this would suggest the Ipswichian stage. The location of zone T-4 above the radiocarbon-dated horizon may be explained by the fact that the passage of glacial ice over the area after 28,140 b.p. led to the incorporation within the till of interglacial deposits similar in age to those still preserved at Teindland. It is not possible to say how near the postulated palaeosol was to the site under investigation. The differences in representation between the similar spectra

Table 1 Pollen assemblage zones

T-6	<i>Pinus</i> – <i>Calluna</i> –Gramineae zone
T-5	<i>Calluna</i> zone
T-4	<i>Alnus</i> –Gramineae– <i>Plantago lanceolata</i> zone
T-3	Gramineae zone
T-2	<i>Pinus</i> – <i>Calluna</i> –Gramineae zone
T-1	<i>Alnus</i> –Gramineae– <i>Plantago lanceolata</i> zone

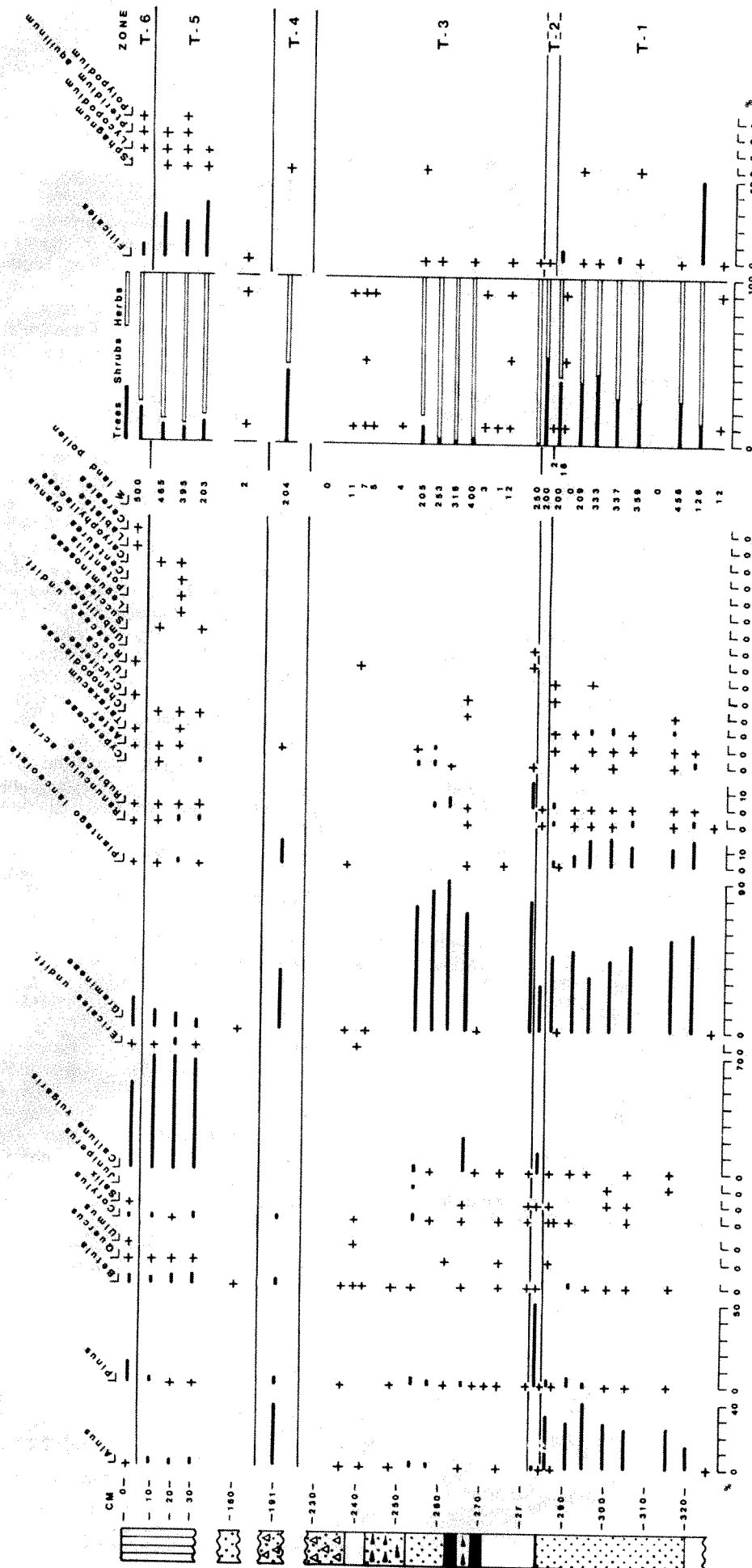


Fig. 2 Pollen diagram from Teindland. + indicates less than two per cent of land pollen sum. Note the differences in the depth scale. Stratigraphic symbols as in Fig. 1.

would result from the spatial disparity between the polleniferous deposits within the interglacial landscape, and possibly differential preservation.

The vegetation at this stage of the posited interglacial would appear to have been light woodland dominated by *Alnus*, the latter especially in the wetter areas. Pine may have been present though the low frequencies of less than 6% might suggest long distance wind transport. This agency or differential pollen destruction⁶ might also be evoked for the traces of *Betula*, *Quercus*, and *Corylus pollen*. The open areas of the landscape would seem to have been dominated by Gramineae, *Plantago lanceolata*, and ferns. The assemblage is somewhat unusual, and this may be in part a function of pollen preservation, though the dominants in zone T-1 are known from proposed Ipswichian deposits in East Anglia^{7,8}. Conformity with the English pattern would not necessarily be expected but the spectra in zone T-1, especially with regard to the *Alnus* and *Plantago lanceolata* frequencies, bare a striking resemblance to the pollen content of the zone Ip III deposits at Swanton Morley in Norfolk, which have been assigned to an "Ipswichian late-temperate zone" immediately preceding an "Early Devensian zone"⁹. The homogeneous spectra of zone T-1 are possibly due to downwash of grains from an interglacial soil surface which may have been at any level below or within the supposed soliflucted horizons of zone T-3.

The lone spectrum of zone T-2 might be interpreted as depicting a later phase of the interglacial. It quite possibly reflects the vegetational conditions pertaining at the time of podsolisation and might be compared to a certain extent with the present-day plant cover of *Pinus sylvestris*, *Calluna vulgaris* and Gramineae spp. overlying the semi-podsol developed above the till. This change between zones T-1 and T-2 may indicate a retrogressive vegetation succession¹⁰ as has been noted in palynological investigations of podsol profiles elsewhere¹¹. The sharp transition between zones T-1 and T-2 (and, indeed T-3) might appear enigmatic. The boundary between T-1 and T-2 occurs at a stratigraphic break where the thin ferric horizon separates yellow and grey sand. Whether this has any implications in terms of deposit age or pollen accumulation or is merely a co-incidental feature arising from the podsolisation process is unclear. Signs of microfossil movement within this section of the profile are not apparent; the spectra are quite distinct with no obvious signs of homogenisation. The question of pollen fixation with abrupt changes between horizons, as opposed to translocation, downwash and homogenisation of pollen in leached mineral profiles, is a subject of long and continuing debate^{5,12}. The Teindland site thus far discussed would appear to provide evidence for the operation of both processes at different times in the development of the pollen profile.

The sample at the base of zone T-3 (281 cm) with its 97.6% herbaceous pollen consisting mainly of Gramineae and Rubiaceae spp., may indicate the cold climatic conditions at the end of the proposed interglacial stage. Alternatively, it possibly belongs chronologically to the polleniferous section in the middle zone T-3 among the soliflucted organic layers. Here is also to be found a predominance of open land taxa. Gramineae pollen dominates throughout with high frequencies of *Calluna vulgaris* pollen at 265 cm and some *Alnus*, *Corylus* and *Juniperus* pollen at 253 cm. It is possible that the representation of these types is due to a warming of local climatic conditions, though some of the pollen may be derived from the pollen within the till by downwash through the unpodsolised sand above the organic layers. Another possible mechanism could be wind transport, an agency which might be particularly evoked for the low frequencies of *Pinus* pollen. The traces of pollen found within and on either side of these probable interstadial spectra may represent some derived

types or be the result of poor pollen preservation. The microflora of zone T-3 indicate cold climatic conditions. This would be consistent with the Middle Devensian radiocarbon date of 28,140 BP, and might be compared with the more oceanic interstadial conditions inferred from the site at Tolsta Head, Island of Lewis, where a polleniferous limnic deposit yielded a radiocarbon date of 27,333 ± 240 BP (SSR-87)¹³.

The interpretation of the palynologically-derived data presented above, corroborates FitzPatrick's stratigraphic suppositions¹ and would seem to provide evidence for the existence of, and conditions within the Middle Devensian and Ipswichian stages in north-east Scotland. Only two polleniferous deposits of pre-Late Devensian age have been reported from Scotland^{13,14}. The importance of the Teindland site in providing possible evidence for the first Scottish Ipswichian stage flora and only the second Middle Devensian microfossil assemblage will be obvious.

In addition, detailed stratigraphic analysis of the till using fabric, particle size and shape criteria has been carried out, demonstrating a glacial origin and probable correlation with the last full glacial conditions in this area. A preferred orientation of pebbles of 110°–120°, accords with the accepted direction of ice flow derived independently from a study of ice directed landforms and melt-water channels¹⁵. This finding tends to contradict a report that the till horizon at Teindland is a soliflucted deposit¹⁶ and adds further weight to FitzPatrick's original interpretation¹, since the deposit has none of the sedimentological characteristics usually associated with soliflucted till, and orientation measurements do not indicate slope controlled deposition. The particle size and shape statistics are similar to results obtained from other sections of the same till in north-east Scotland (Chester unpublished). It has been argued that there is no evidence for more than one phase of glaciation in north-east Scotland and that all till deposits are of the same age^{15,17,18}. If this reasoning is accepted, then the Teindland site provides a maximum age for the onset of full glacial conditions in this area.

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Geography and dispersal of Galapagos Islands vascular plants

NEARLY every naturalist who has dealt with the Galapagos Islands since Darwin¹ has pointed out the flora's obvious geographical affinities with tropical America, stressing relationships with South America, Mexico, Central America and the West Indies. But as the tropical American flora has become better known, most Galapagos species once thought to be only Mexican, Central American or West Indian in their extra-Galapagean distributions also have been found to occur in northern and western tropical South America (for example, the widespread *Zanthoxylum fagara* (L.) Sarg., Rutaceae) or to have been incorrectly identified (for example, the endemic *Chamaesyce viminea* (Hook. f.) Burch, Euphorbiaceae). Svenson² first indicated the flora's lack of affinity with Mexico and Central America. The study reported here is the first to present evidence that West Indian relationships do not exist. After publication of the *Flora of the Galapagos Islands*³ and several subsequent papers⁴⁻¹², it is now also possible to quantify the geographical relationships of the vascular flora and to study the plants' dispersal mechanisms. This study shows the

endemics have their closest known relatives in South America as well. The geographical evidence is overwhelming that the indigenous Galapagos flora has been derived almost totally from South America, most probably from the Andean region. With only eight exceptions, all non-endemic species or the presumed progenitors of the endemics occur in this region.

How did the plants get there? As a result of this study, I estimate that a minimum of 378 original introductions of plant disseminules can account for the 522 indigenous vascular plants of the archipelago (Table 2).

It is no surprise that these oceanic islands have received the bulk of their flora by means of long-distance dispersal through the agency of birds, with fruits, seeds or vegetative disseminules either attached externally or carried internally. The importance of long-distance bird dispersal of disseminules to oceanic islands is well documented^{13,14}. I estimate that 144 original introductions (64%) arrived internally (for example, most Cyperaceae), 34 (15%) in mud attached to birds (for example, *Azolla microphylla* Kaulf., Azollaceae), 28 (12%) attached by viscid structures of seeds or fruits (for example, *Eliptia alba* (L.) Hassk., Asteraceae) and 19 (8%) attached mechanically (for example, *Bidens riparia* HBK., Asteraceae).

Hooker¹⁵, Robinson¹⁶, and Stewart^{17,18} indicated the prob-

Table 1 Geographical relationships of the indigenous vascular plants of the Galapagos Islands

	Endemic	Neotropical	Pantropical	Andean	Mexico and Central America	South America	Total
Pteridophytes	8	52	14	15			
Monocotyledons	20	38	22	3		2	91
Dicotyledons	208	65	26	43	4	2	83
Total	236 (45%)	155 (30%)	62 (12%)	61 (12%)	4 (1%)	4 (1%)	522

Geographical areas are defined as follows: endemic (occurring only in the Galapagos Islands); neotropical (distributed generally in the American tropics); pantropical (distributed in both the Old and New World tropics); Andean (occurring only in western South America from Venezuela to Chile, generally or in part); Mexico and Central America (occurring only in Mexico and/or Central America); South America (occurring only in extra-tropical South America).

flora's relationships to be with adjacent South America and that birds have had the most important role in plant dispersal to the islands.

The known extra-Galapagean distributions for the archipelago's indigenous vascular plants (that is, those not introduced by man's activities) are given in Table 1, which includes the absolute numbers and percentages of the vascular flora's distribution by geographical region. The numbers of taxa include all species, subspecies and varieties, but forms are excluded. A complete list of taxa and discussion of each will be published elsewhere. Further refinements in the knowledge of extra-Galapagean distributions may alter these figures slightly.

Ninety-nine per cent of the non-endemic vascular plant taxa in the Galapagos Islands also occur in South America. The remaining 1% occurs in Mexico and Central America. All but four of 286 indigenous non-endemic taxa in the islands are known from the land area nearest the archipelago, which at its closest is about 800 km away. The

ability that much of the flora was derived in this way, although they presented little data to support this viewpoint. Seabirds have been hypothesised as being instrumental in the early colonisation of the archipelago by other organisms¹⁹. Both seabirds and shorebirds have played an important role in plant dispersal, with migrants and casual visitors perhaps the most important. "More species have been recorded as migrants or vagrants than breed within the islands, and during the northern winter migrant birds form a very important part of the Galapagos avifauna"²⁰. Many of these are seabirds and shorebirds. The percentage of those disseminules having arrived on or in birds is greater (to 77%) when flowering plants only are considered.

In contrast to the figures of Table 2 and those given above are those of Carlquist^{13,14}, which were derived from Stewart's flora¹⁷ and based on an estimate of 308 original introductions. Carlquist determined that the flora had been derived as follows: 73% by birds (27.7% internal, 22.8% mechanical attachment, 13.7% in mud, 8.5% viscid attach-

Table 2 Original introductions that have resulted in the present vascular plant flora of the Galapagos Islands

Introduced	Birds	Man	Wind	Oceanic drift	Total
Pteridophytes	1		86		87
Monocotyledons	58	38	14	2	112
Dicotyledons	166	143	18	33	360
Total	225 (40%)	181 (32%)	118 (21%)	35 (6%)	559
Total for natural introductions	225 (60%)		118 (31%)	35 (9%)	378

ment); 23% by drift (21.8% frequent drift, 1.3% rare drift or rafting), and 4% by wind.

His figures and mine are close for overall dispersal by birds, but they are reversed for wind dispersal and drift. Although floating vegetation originating on the mainland has been reported from the vicinity of the archipelago²¹⁻²³, or even cast up on its beaches^{18,24}, drift has had a minor role in plant colonisation of the Galapagos. Those species that have arrived in this way are all members of the mangrove, beach or salt flat associations. I can find no evidence for any vascular plant species having arrived by rafting.

The small number of species that have been derived through oceanic drift is not surprising. Although drift-dispersed species have relatively high immigration rates in comparison with those which are dispersed by birds and wind, very few species are adapted to this mode of introduction. In addition, their higher immigration rates and the small numbers of habitats available to them in the Galapagos also reduce the opportunities for endemism in this group²⁵. The role of man in the introduction of exotic species into the islands is surprising (Table 1). Even Hooker¹⁹ remarked on the number of alien species that man had added to the flora by the time of Darwin's visit in 1835. Man has introduced 124 weeds by the latest count, and 57 of his cultivated exotics have escaped and now reproduce themselves in the wild. Today *Homo sapiens* has replaced birds as the most important factor in the dissemination of plants to the Galapagos. The deleterious effects on the native flora of some of the species introduced by man are just beginning to be appreciated^{6,19,26-28}.

Recent potassium-argon studies²⁹ indicate that the archipelago has a probable maximum age of 3 Myr. This means that the arrival and establishment of one successful disseminule about every 7,900 yr would account for the present indigenous flora. Introduction and subsequent extinction cannot be estimated, but they certainly have occurred as well. This is a higher rate of introduction than Fosberg's³⁰ estimate of one successful disseminule every 20-30,000 yr to account for the derivation of the indigenous vascular flora of the Hawaiian Islands, but the latter are much further from their source areas than are the Galapagos.

Such a relatively high rate of introduction is not surprising. These volcanic islands are a jumble of open pioneer habitats inhibited by a weedy flora. As others have pointed out, dispersal is only half the battle; establishment is the other half. In the Galapagos, those plants that have become established are almost all weedy, a phenomenon first recognised by Darwin¹. The chance of immigrants surviving in any specific locality is greater initially than later when more closed communities have evolved. Weedy plants, being adapted to open habitats, have been at an advantage when their disseminules reached the Galapagos Islands. Their offspring have given us the present flora.

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Hearing lips and seeing voices

MOST verbal communication occurs in contexts where the listener can see the speaker as well as hear him. However, speech perception is normally regarded as a purely auditory process. The study reported here demonstrates a previously unrecognised influence of vision upon speech perception. It stems from an observation that, on being shown a film of a young woman's talking head, in which repeated utterances of the syllable [ba] had been dubbed on to lip movements for [ga], normal adults reported hearing [da]. With the reverse dubbing process, a majority reported hearing [bagba] or [gaba]. When these subjects listened to the soundtrack from the film, without visual input, or when they watched untreated film, they reported the syllables accurately as repetitions of [ba] or [ga]. Subsequent replications confirm the reliability of these findings; they have important implications for the understanding of speech perception.

To further confirm and generalise the original observation, new materials were prepared. A woman was filmed while she fixated a television camera lens and repeated ba-ba, ga-ga, pa-pa or ka-ka. Each utterance was repeated once per second, with an interval of approximately 0.5 s between repetitions. From this master recording four dubbed video-records were prepared in which the original vocalisations and lip movements were combined as follows: (1) ba-voice/ga-lips; (2) ga-voice/ba-lips; (3) pa-voice/ka-lips; (4) ka-voice/pa-lips. Dubbing was carried out so as to ensure, within the temporal constraints of telerecording equipment, that there was auditory-visual coincidence of the release of the consonant in the first syllable of each utterance. Each recording comprised three replications of its auditory-visual composite. Four different counterbalanced sequences of recordings (1)-(4) were prepared, each with a ten-second gap of blank film between successive segments. The recordings were suitable for relay via a 19-inch television monitor; audio-visual reproduction was of good quality.

Twenty-one pre-school children (3-4 y), 28 primary school children (7-8 yr) and 54 adults (18-40 yr) were tested. The adult sample was predominantly male; there were approximately equal numbers of boys and girls in the younger samples. Subjects were individually tested under two conditions: (1) auditory-visual, where they were instructed to watch the film and repeat what they heard the model saying, and (2) auditory only, where they faced away from the screen and again had to repeat the model's utterances. Every subject responded to all four recordings ((1)-(4) above) under each condition, each time in a different sequence; sequence of presentation was counterbalanced across subjects.

For the purpose of analysis, a correct response was defined as an accurate repetition of the auditory component of each recording. Under the auditory-only condition accuracy was high, with averages of 91, 97 and 99% for pre-school, school age and adult subjects respectively; errors were unsystematic. Under the auditory-visual condition, where subjects heard the original soundtrack, errors were substantial. For pre-school subjects

Table 1 Stimulus conditions and definition of response categories from auditory-visual condition

Stimuli		Response Categories				
Auditory component	Visual component	Auditory	Visual	Fused	Combination	Other
ba-ba	ga-ga	ba-ba	ga-ga	da-da	—	—
ga-ga	ba-ba	ga-ga	ba-ba	da-da	gabga bagba baga gaba	dabda gagla etc.
pa-pa	ka-ka	pa-pa	ka-ka	ta-ta	—	tapa pta kafta etc.
ka-ka	pa-pa	ka-ka	pa-pa	—	kapka pakpa paka kapa	kat kafa kakpat etc.

average error rate was 59%, for school children 52% and for adults 92%.

Subsequent analysis was confined to detailed consideration of responses to the auditory-visual presentations. Responses were first categorised according to the operational definitions illustrated in Table 1.

The meaning of 'auditory' and 'visual' categories is self-evident. A 'fused' response is one where information from the two modalities is transformed into something new with an element not presented in either modality, whereas a 'combination' response represents a composite comprising relatively unmodified elements from each modality. Responses which could not be unambiguously assigned to one of these four categories were allocated to a small, heterogeneous 'other' category. Table 2 presents the percentage of responses in each category.

Table 2 shows that the original observation of the effect of [ba]/[ga] presentations on adult responses is highly replicable; 98% of adult subjects gave fused responses to the ba-voice/ga-lips presentation and 59% gave combination responses to its complement. The effect is also generalisable, at least to other stop consonants; 81% of adults gave a fused response to pa-lips/ka-voice and 44% gave combination responses to its complement. The effects, however, are more pronounced with [ba]/[ga] than with [pa]/[ka] combinations; the latter comment applies to all ages.

The data in Table 2 also illustrate that the auditory perception of adult subjects is more influenced by visual input than is that of subjects in the two younger groups; the latter do not differ consistently from each other. It is interesting to note that where responses are dominated by a single modality, this tends to be the auditory for children and the visual for adults. However, it should also be noted that the frequency of fused responses to ba-voice/ga-lips, and pa-voice/ka-lips presentations is at a substantial level for

pre-school and school children alike. These auditory-visual illusions, therefore, are observable across a wide age span, although there clearly are age-related changes in susceptibility to them, particularly between middle childhood and adulthood.

Appropriate analyses confirm that the various effects reported for the auditory-visual condition are statistically significant. Alone, however, the data fail to testify to the powerful nature of the illusions. We ourselves have experienced these effects on many hundreds of trials; they do not habituate over time, despite objective knowledge of the illusion involved. By merely closing the eyes, a previously heard [da] becomes [ba] only to revert to [da] when the eyes are open again.

Contemporary, auditory-based theories of speech perception are inadequate to accommodate these new observations; a role for vision (that is, perceived lip movements) in the perception of speech by normally hearing people is clearly illustrated. Our own observations and those of others¹ indicate that, in the absence of auditory input, lip movements for [ga] are frequently misread as [da], while those for [ka] are sometimes misread as [ta]; [pa] and [ba] are often confused with each other but are never misread as [ga, da, ka or ta]. It is also known that, in auditory terms, vowels carry information for the consonants which immediately precede them². If we speculate that the acoustic waveform for [ba] contains features in common with that for [da] but not with [ga], then a tentative explanation for one set of the above illusions is suggested. Thus, in a ba-voice/ga-lips presentation, there is visual information for [ga] and [da] and auditory information with features common to [da] and [ba]. By responding to the common information in both modalities, a subject would arrive at the unifying percept [da]. Similar reasoning would account for the [ta] response under pa-voice/ka-lips presentations.

By the same token, it could be argued that with ga-voice/ba-lips

Table 2 Percentage of responses in each category in the auditory visual condition

Stimuli		Subjects	Responses		Combination	Other
Auditory	Visual		Auditory	Visual		
ba-ba	ga-ga	3-5 yr (n=21)	19	0	81	0
		7-8 yr (n=28)	36	0	64	0
		18-40 yr (n=54)	2	0	98	0
ga-ga	ba-ba	3-5 yr (n=21)	57	10	0	14
		7-8 yr (n=28)	36	21	11	0
		18-40 yr (n=54)	11	31	0	4
pa-pa	ka-ka	3-5 yr (n=21)	24	0	52	24
		7-8 yr (n=28)	50	0	50	0
		18-40 yr (n=54)	6	7	81	6
ka-ka	pa-pa	3-5 yr (n=21)	62	9	0	24
		7-8 yr (n=28)	68	0	0	0
		18-40 yr (n=54)	13	37	0	6

and ka-voice/pa-lips combinations the modalities are in conflict, having no shared features. In the absence of domination of one modality by the other, the listener has no way of deciding between the two sources of information and therefore oscillates between them, variously hearing [babga], [papka], and so on.

The *post facto* nature of this interpretation is acknowledged. More refined experimentation is required to clarify the nature and ontogenetic development of the illusions and their generality needs further investigation. We are at present working on these issues but believe that the finding now reported are of some interest in their own right.

Full details of the dubbing procedure are available from us. We thank the staff of the University of Surrey AVA Unit for their technical assistance in preparing materials and also Susan Ballantyne whose lip movements we filmed.

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Squeezing speech into the deaf ear

DEAFNESS caused by attenuation at the middle ear—conduction deafness—can be ameliorated by hearing aids and surgical procedures, but damage to the cochlea presents more severe problems¹. We are altering the amplitudes of different parts of the speech wave relative to one another, so that the features normally determining intelligibility are selectively amplified. Tests using speech modified in this way will show whether major aspects of speech processing are unchanged by sensorineural deafness, and, if so, whether the improvement is large enough to justify the use of such processing in hearing aids.

The principal characteristics of sensorineural deafness due to cochlear damage are: (1) loss of sensitivity to high frequencies; (2) tinnitus—whistling or hissing sounds; (3) recruitment—reduction of dynamic range for intensity, the threshold for detection being raised while the maximum acceptable intensity is near normal. Both conduction and sensorineural deafness can be simulated in the normal ear^{2,3}; conduction deafness by ear plugs and sensorineural deafness by adding masking white noise (that is, noise containing equal power in all audible frequencies). Linear amplification will compensate for the signal attenuation of conduction deafness. It cannot, however, overcome loss of hearing associated with the limited dynamic range of the damaged cochlea. Standard hearing aids applied in the latter case can amplify the peak sound intensities above acceptable limits, overloading the middle ear to produce distortion, discomfort and sometimes pain without giving adequate intelligibility to normal speech. They may also produce further damage to the hair cells^{4,5}.

Fortunately, the energy peaks are not important as carriers of speech information⁶. Most of the speech information is carried by the zero-energy crossover points of the wave. This implies that the form of the speech wave is not inherently important, and that liberties can be taken with the speech wave for a hearing aid. Gregory and Wallace⁷ concluded that it should be possible to prevent overloading while retaining the necessary information, by using suitable peak clipping in hearing aids. Amplitude clipping was, however, found (by ourselves and others) to produce unpleasantly distorted sounds, and the Fourier components generated by sharp clipping give masking tones which reduced intelligibility. "Rounding" the flat tops

of the clipped waves with a high cut filter gives some improvement, but removes information since some of the generated components are in the speech frequency band. Alternatives, such as frequency transposition, were also attempted but were abandoned for various reasons⁸. Amplitude compression (AGC) is standard practice for broadcasting and recording. It is only suitable for hearing aids if the "attack time" is made short enough to limit individual energy peaks (of the order of hundreds of microseconds) and so minimise peak overloading. The "release time" must also be made short enough to preserve information of consonants following vowels without causing sound level fluctuations ("breathing")⁷.

Villchur used a 2-channel amplitude compressor with an attack time of "less than 1ms" and a release time of 2000 dB s⁻¹. He found improvements of syllable recognition of from 22% to 160% in quiet and 10% to 230% in a "cocktail party" situation. "Improvement was defined as

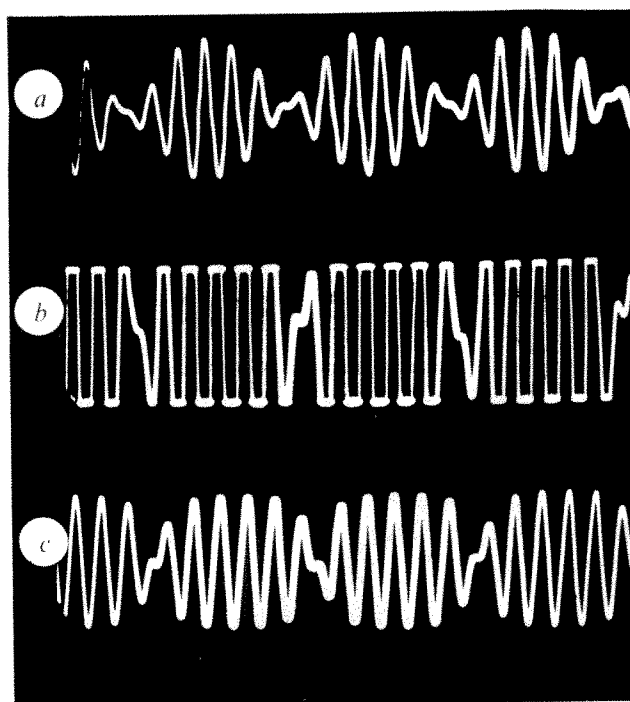
$$100 \left(\frac{\text{score with processing} - \text{score without}}{\text{score without processing}} \right)$$

An alternative and possibly complementary approach is instantaneous amplitude clipping, but without the squaring and generation of cross modulation products in the speech band associated with peak clipping. This has for a long time seemed impossible; but it can now be achieved.

The solution we have adopted is to generate the inevitable cross-modulation products outside the speech band. This is done by amplitude modulating a high frequency carrier with the speech, and clipping the carrier^{8,9}. The cross-modulation products are now multiples of the chosen carrier frequency. By choosing a suitable carrier frequency (50 kHz-100 kHz) the harmonics and cross-modulation products generated by the clipping are widely separated from the speech band, and so can be removed. The result is instantaneous limitation of the audio peaks without flattening or appreciable intermodulation product distortion.

Considering first normal audio frequency peak clipping, any wave exceeding the clipping level will be flattened. For two

Fig. 1 Unprocessed and processed wave forms, from "beating" a pair of AF sine waves. *a*, The unprocessed wave. *b*, Processed by audio frequency peak clipping. *c*, Processed by the carrier clipping system.



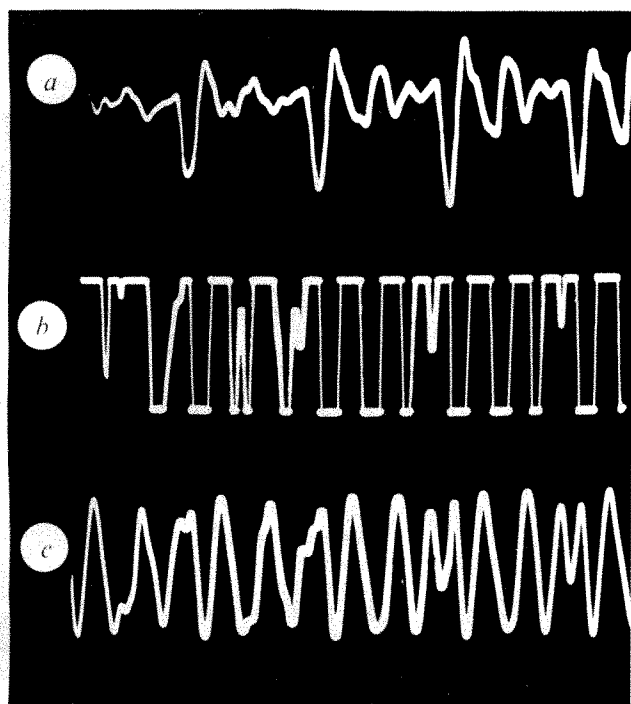


Fig. 2 Unprocessed and processed wave forms of speech. *a*, Unprocessed speech wave. *b*, Processed by audio frequency peak clipping. *c*, Processed by the carrier clipping system. It will be seen that the carrier clipping system retains the wave form while limiting the amplitude. In all cases the peak amplitudes are equal though the small signal amplification is increased in the *b* and *c* waves. The wave form is destroyed with audio frequency clipping (*b* waves) while the wave form is retained by the carrier clipping (*c* waves).

equal amplitude sine waves, with frequencies f_1 and f_2 respectively, the intermodulation products will include

$$(f_1 + f_2)/2 + n(f_1 - f_2)/2 \\ \text{for } n = 3, 5, \dots$$

There will also be another overlapping series of products centred on harmonics of f_1 and f_2

$$m(f_1 + f_2)/2 + nm(f_1 - f_2)/2 \\ \text{where } n = 1, 2, 3, \dots \\ m = 2, 3, 4, \dots$$

With a complex speech wave, there will be corresponding cross-modulation products for each component frequency and its Fourier terms. It is indeed remarkable that audio frequency clipped speech is at all intelligible.

Consider now a single side band suppressed carrier (s.s.b.) signal. If this is clipped, much the same products are produced as in the audio frequency case just considered; but now the intermodulation products and harmonics are widely separated from the signal.

Taking signals $F_1 = 1$ kHz and $F_2 = 2$ kHz and a carrier frequency of 50 kHz then f_1 and f_2 will be 51 and 52 kHz respectively. The modulation products associated with m and n values will be centred on integral multiples of the carrier, 50 kHz. So they are now widely separated from f_1 and f_2 and can be filtered out. The clipped s.s.b. signal is now heterodyned back to the original audio frequencies. The frequencies left will be F_1 and F_2 .

Harmonic frequencies produced by the clipping are lost to the audio band. The clipped waves are not flat topped; but are limited in amplitude with virtually no harmonic distortion. The wave forms are shown in Figs 1 and 2.

Children and adults suffering sensorineural loss as diagnosed clinically have been tested with carefully calibrated equipment in the laboratory. The children attend the Partially Hearing

Unit of Hengrove School, Bristol. Some of the adults were patients at the Bristol Royal Infirmary. Normal subjects were also tested, with added white noise to simulate sensorineural deafness. Comparison was made between three types of speech processing. Speech from pre-recorded test tapes was processed by each of three systems and fed into the subject's ears by headphones. The three processing systems were: (1) linear amplification; (2) audio frequency peak clipping; (3) high frequency carrier clipping (HFCC). In all cases the audio frequency band was restricted to 400 Hz–2.5 kHz, and 16 dB of clipping was used both for audio frequency peak clipping and high frequency peak clipping, that is, the signal was amplified by 16 dB then reduced to the original level by clipping. The apparatus was set up by using a 900 kHz pure tone to equate the output levels for the three systems. The output from the tape recorder was adjusted so that the peak levels of the test words coincided with the level of the calibration tone. The amplification for each of the patients was adjusted for about fifty per cent recognition, using Fry's single word and sentence test tapes. The level for the normal subjects was set so that the calibration tone was 80 dB above the average pure tone threshold, and broad band masking noise of 50 dB (for word tests) or 60 dB (for sentences) was added, after signal processing to simulate sensorineural deafness. Each test lasted about one hour and included about 350 individual words, or 175 test sentences.

The results are summarised in Fig. 3. Most subjects showed an improvement of word recognition with HFCC. This varied from +145% down to -35%.

The scores are shown as

$$100 \left(\frac{\text{word score with HFCC} - \text{word score with linear processing}}{\text{word score with linear processing}} \right)$$

This is the same method of data presentation as used by Villchur.

The most conspicuous difference between the groups of subjects was that the variability was much greater for those with impaired hearing than for normal subjects with noise masking. A similar result was obtained with sentence tests (Fig. 4). Peak clipping was less effective than HFCC.

These results were obtained without practice. No consistent learning effect was observed within a test. An attempt will be made to measure any learning effect which does occur over a timescale of several weeks.

Speech which is amplitude limited in this way sounds almost normal. With equated peak amplitudes it sounds considerably (up to about 10 dB) louder. Intelligibility is increased. Its main advantage in nerve deafness is its ability to amplify the information-bearing features of the speech wave while limiting the peak energies to a safe value. This reduces or removes intermodulation products generated by overloading the mechanism of the middle ear, and protects the cochlea from unnecessary damage.

Six subjects (2 deaf, 4 normal with noise masking) were tested with amplitude compression using the same limiting ratio, with an attack time of 0.25 ms and a release time of 25 ms. The results were comparable to those of Villchur⁷.

For a practical hearing aid, the entire dynamic range of sounds reaching the aid should be considered and the output must remain tolerable in overload conditions. Special consideration should be paid to the energy and frequency ranges of sounds important to the user, especially speech. For normal ears, recognition of speech is about 50% at 20 dB above pure tone threshold. The normal dynamic range of speech is about 60 dB, with a mean of about 40 dB. The upper mean limit considered safe in industry is 90–100 dB and distortion for speech becomes significant at peak energies of about 120 dB. This implies that for the normal ear there is a very small, if any, margin for linear amplification of speech before risk of damage. This applies also to sensorineural deafness, with perhaps more risk of further damage to the impaired cochlea. These

figures imply that an aid suitable for sensorineural deafness should reduce the dynamic range of speech proportionately to the rise in pure tone threshold. This could require as much as 80 dB compression which is difficult to achieve by normal (a.g.c.) compression. For the HFCC system 80 dB compression is possible but gives considerable distortion. A hybrid system could well be adopted, with say 40 dB slow acting a.g.c. compression and 40 dB instantaneous HFCC peak limiting.

According to the prevailing signal level, the HFCC system operates in three modes: (1) for low intensities it is a linear amplifier; (2) for intermediate intensities it gives linear amplification to the low energy components while limiting the high energies; (3) very high signal energies will overload early stages of the system, producing harmonic components which will however be limited in amplitude. We are primarily concerned with the middle energy range, where the aid is limiting without significant distortion. The increase in intelligibility we have found was for peak energies well within the dynamic range of the ear. Where high energies are required the value of the system can be greater—to the point where speech can be

Fig. 3 Percentage improvement of word identification with high frequency carrier clipping. *A*, Pupils at Hengrove Partial Hearing Unit; *B*, patients at Bristol Royal Infirmary; *C*, other adults with impaired hearing; *D*, control group; unimpaired hearing, white noise masking.

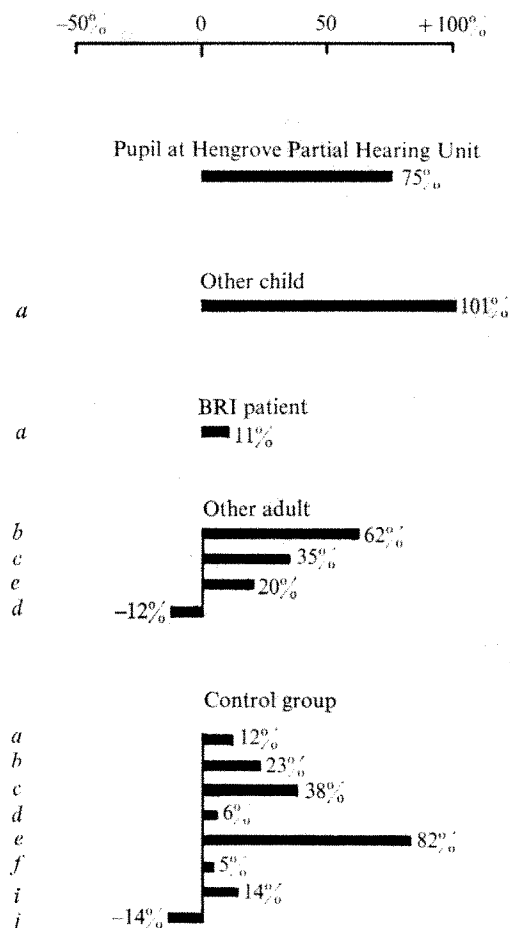
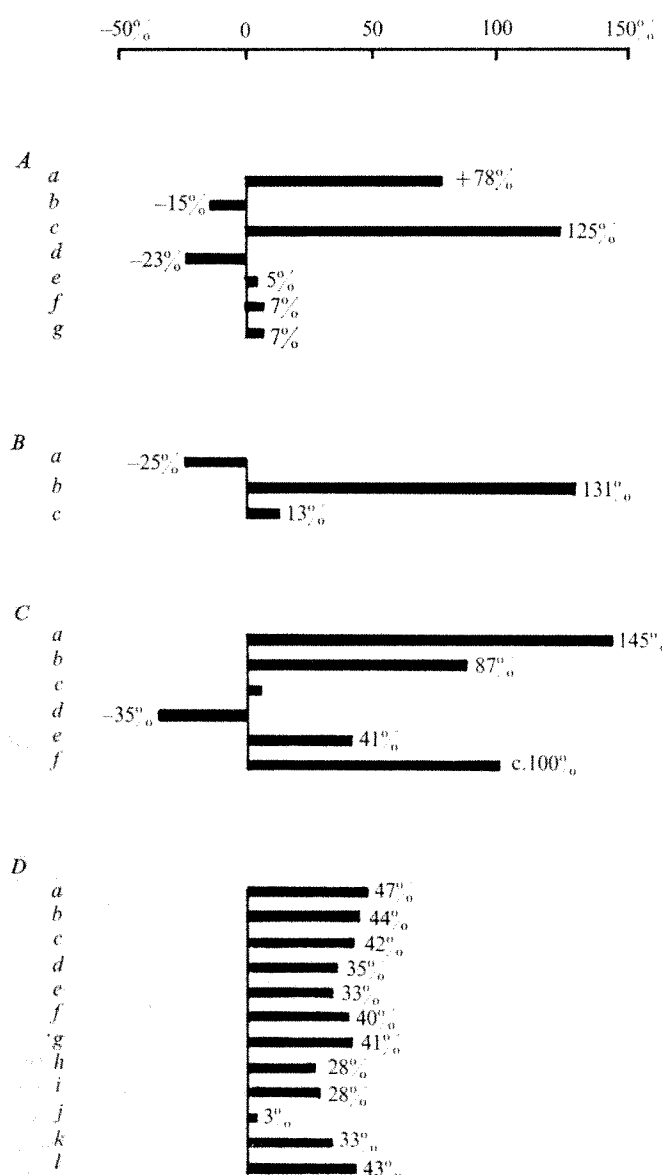


Fig. 4 Percentage improvement of sentence identification with high frequency carrier clipping.

provided by the limiting system but only with extreme discomfort or not at all by normal linear amplification. Very severely handicapped children will, however, require training to accept speech without concomitant lip reading even if the signal is adequate after processing.

Although a few years ago the complexity required would have made a device such as this impracticable, recent advances in electronic circuits and falling cost by no means rule it out as a practical hearing aid. The system has a further advantage over conventional aids: "howl-round" restricts their usable amplification, especially for children whose ear moulds tend to fit loosely. HFCC limits the howl-round energy to a safe, and not unpleasant, limit. We are working on fail-safe methods of avoiding this acoustic disaster.

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Right hemispheric sensitivity for the McCollough effect

THE mammalian cerebral cortex is divided into two hemispheres and it seems in man that each half specialises in a different kind of information processing. Linguistic skills are, for the most part, localised in the left hemisphere while the right may dominate in visual/perceptual tasks¹⁻⁴.

This division of labour has important theoretical and clinical implications but its basis is not understood. However, I have found that pink McCollough effect hues, perceived by subjects after exposure to a green square-wave grating, are reported to be stronger in the left visual field even when initially processed by the right cerebral hemisphere. Since the McCollough effect seems to be a process related to the orientation specific neurones of the striate cortex⁵, this suggests a specialisation for visual perception in the right cerebral hemisphere which may include the earliest visual areas.

Neurones in the striate cortex (area 17) of the primate occipital lobe are the first cortical units to receive retinal information. Electrical recordings from these cells indicate that each may respond only to a specific pattern of light and dark. A cortical neurone, for example, might be sensitive to lines of a certain orientation, width, colour and/or which move in a specific direction⁶. It has been postulated that the superiority of the right hemisphere in some visual tasks, involving orientation⁷ and brightness judgments⁸, could be due to hemispherical differences between the striate areas^{1,9,10}.

A direct test of the hypothesis that the differentiation in hemispherical information extends to the primary input areas of the cortex is difficult. Electrode recordings from human cortical neurones give limited information, but psychophysical techniques are available to tap these cortical populations⁹, and can be used to exploit the phenomenon known as the McCollough effect⁹. If a subject views vertically oriented green and black bars (Fig. 1a) for several minutes, a similar vertical black and white grating will seem pink. If the orientation or bar width of the grating are varied, this complementary after-effect will weaken. These hues can be made contingent on orientation, width (spatial frequency) or motion which correspond to the selectivities of striate neurones⁹⁻¹¹.

These pattern contingent hues are best explained by a lowered sensitivity in cortical units tuned to both colour and the spatial configuration of the adaptation stimulus^{9,12}. For example, exposure to green vertical lines would adapt out neurones responsive to green vertical. The long duration of the effect suggests that it may be related to learning or memory^{13,14}.

Furthermore, the McCollough effect is orientation and movement selective and must not occur below the primary cortex. Second, the effect does not easily transfer from one eye to the other^{9,13,15-17}. This suggests an early striate locus as this area seems to be more monocular than later cortical regions¹⁸⁻²⁰.

I have tested the strength of the McCollough effect in the left compared with the right cortical hemisphere. Because of the anatomy of the visual pathways, stimuli presented to the left of a fixation point are transmitted to the right hemisphere and those on the right reach the left hemisphere. Thus subjects were exposed to a large green and black vertical 7.5 cycle deg⁻¹ of visual angle, square wave grating (Fig. 1a) over which they freely scanned for

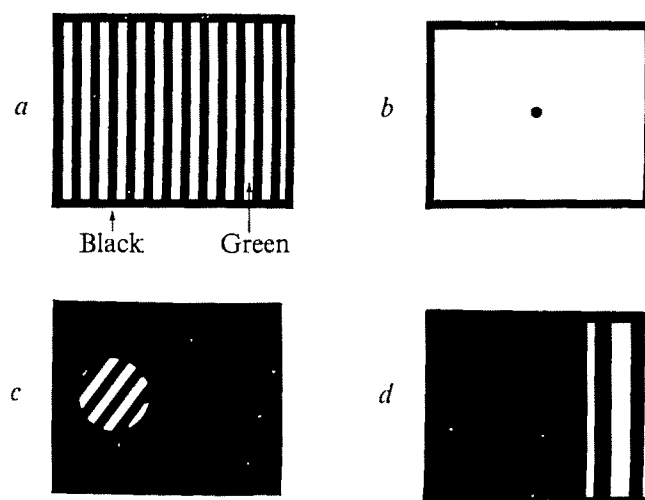


Fig. 1 Descriptions and schematic examples of stimuli. Scale does not exactly correspond with that of the actual slides. *a*, Green vertical adaptation grating (coloured filter = Kodak Wratten 53, contrast ratio = 0.97, mean luminance = 19 cd m⁻², visual angle of slide = 4.5° vertically by 8.25° horizontally, spatial frequency = 7.5 cycles deg⁻¹). With green filter removed, slide served as achromatic test standard (see text). Luminance and contrast were constant for all slides in the experiment. *b*, Fixation point, presented for a 500-ms period before each test patch. *c*, Achromatic 30° test patch for right hemisphere (left visual field). Test patch series orientations were 0°, 10°, 20°, 30°, 40° and 50° off vertical. Grating diameter = 2.75° with centre 2.1° from fixation. Total series consisted of 12 slides, 6 with patch to the left of fixation and 6 to the right. *d*, Achromatic +1 octave spatial frequency test patch for left hemisphere (right visual field). Test patch series spatial frequencies varied -2, -1, 0 (7.5 cycles deg⁻¹), +1 and +2 octaves from 7.5 cycles deg⁻¹ of visual angle. Grating patches = 4.5° vertically by 2° horizontally with their innermost edge 1.1° from fixation. Total series consisted of ten slides, five with patches to the left of fixation and five to the right.

250 s. The scanning during adaptation was to reduce the formation of patterned negative afterimages. The adaptation period should produce a pink aftereffect in both visual fields. Small black and white test gratings were then presented for 90 ms to the left or right of fixation (Fig. 1b). These conditions prevented eye movements and assured initial stimulus presentation to the appropriate hemisphere. The strength of the hues was determined as the target gratings varied in spatial frequency and orientation.

It might be expected that if the right hemisphere is perceptually more sensitive or more efficient in some kind of perceptual learning process than the left, the complementary hues would be stronger with the test stimuli presented to the left visual field. The basic idea is to test cortical laterality of function with an after-effect that seems to be produced at the striate cortex.

Three right-handed subjects compared the strength of the McCollough hues in both hemispheres as test patch orientation varied from vertical (0°) in clockwise steps of 10° up to a 50° orientation. (Fig. 1c). After viewing a black and white version (Fig. 1a) of the adaptation grid, the pink perceived on this grid was assigned a value of 10. They were then to rate the hue of the test patches (Fig. 1c) presented to the left or right of fixation on the basis of this "10" value.

Each subject was adapted and tested using the left eye, right eye or both on separate days. Each eye condition was run twice for a total of six sessions with order counter-balanced. On each day the test and adaptation sequence was run five times. After each adaptation period (see text) the test series (Fig. 1c) was run twice with order counter-balanced between and within trials. Eye conditions were not significant and collapsed so that each patch received 60 ratings. The data were transformed by dividing a

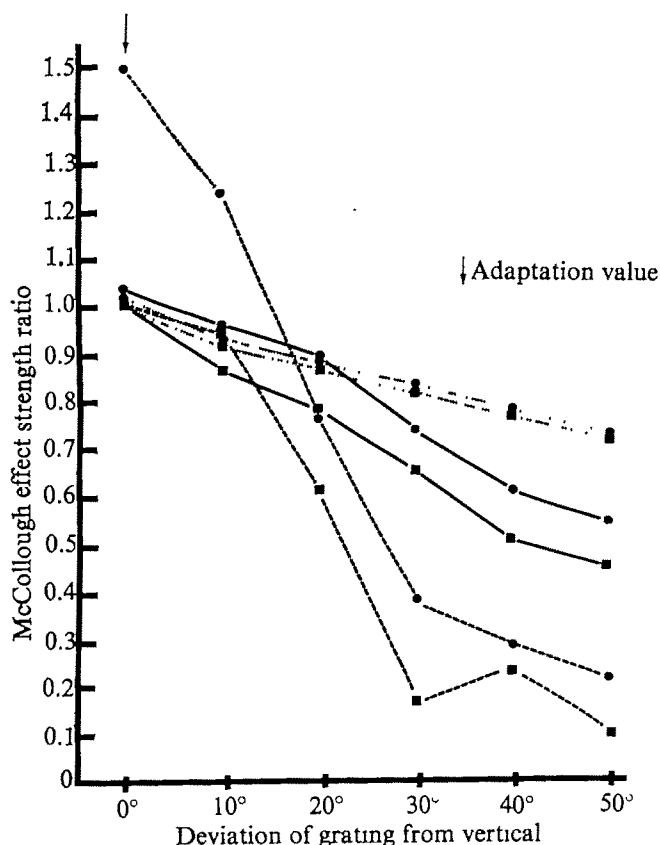


Fig. 2 McCollough effect strength ratios for subjects GL, WE and WH as test grating orientation varied from vertical (0°) as a function of initial cortical hemisphere of stimulus presentation. The vertical orientation used for adaptation is indicated with an arrow. Each point represents 60 ratings (see text). \bullet — \bullet : GL, right hemisphere; \blacksquare — \blacksquare : GL, left hemisphere; \bullet — \bullet : WE, right hemisphere; \blacksquare — \blacksquare : WE, left hemisphere; \bullet — \bullet : WH, right hemisphere; \blacksquare — \blacksquare : WH, left hemisphere.

subject's mean for a particular test orientation and cortical hemisphere by that subject's value for the left hemisphere (right visual field) at 0° (vertical) which was the adaptation orientation. This in no way altered the results of statistical analyses for each subject but only served for convenient graphic representation. For comparison, the untransformed left hemisphere 0° mean ratings for GL, WE and WH were 9.05, 8.27 and 0.95 respectively.

Three subjects were similarly run except that the test patch (Fig. 1d) varied ± 2 octaves in spatial frequency from the adaptation value of 7.5 cycles deg^{-1} . Their data were similarly transformed by dividing the subject's mean for a particular spatial frequency and cortical hemisphere by that subject's value for the left hemisphere (right visual field at 0 octaves; 7.5 cycles deg^{-1}). Again, for comparison, the untransformed left hemisphere 0 octave mean ratings for JC, WH and GL were 4.72, 3.72 and 6.13 respectively.

The ratios for the orientation conditions are presented in Fig. 2. For each subject, the strength of the pink McCollough effect significantly decreased as orientation varied off vertical (GL, WH, WE; $P < 0.001$). Right hemisphere hues were consistently stronger than the left (GL, $P < 0.04$, WH, $P < 0.003$; WE, $P < 0.001$). The spatial frequency data in Fig. 3 are similar. The after-effect decreased as spatial frequency varied from 7.5 cycles deg^{-1} (WH, GL, JC: $P < 0.001$), and the right hemisphere hues were strongest (WH, $P < 0.004$; GL, $P < 0.001$; JC, $P < 0.02$).

The drop in the pink McCollough effect as orientation and spatial frequency varied off the adaptation value is typical and indicates a cortical locus^{9,10,12}. There are three possible explanations for the stronger right hemisphere hues: (1) degradation of information quality during trans-

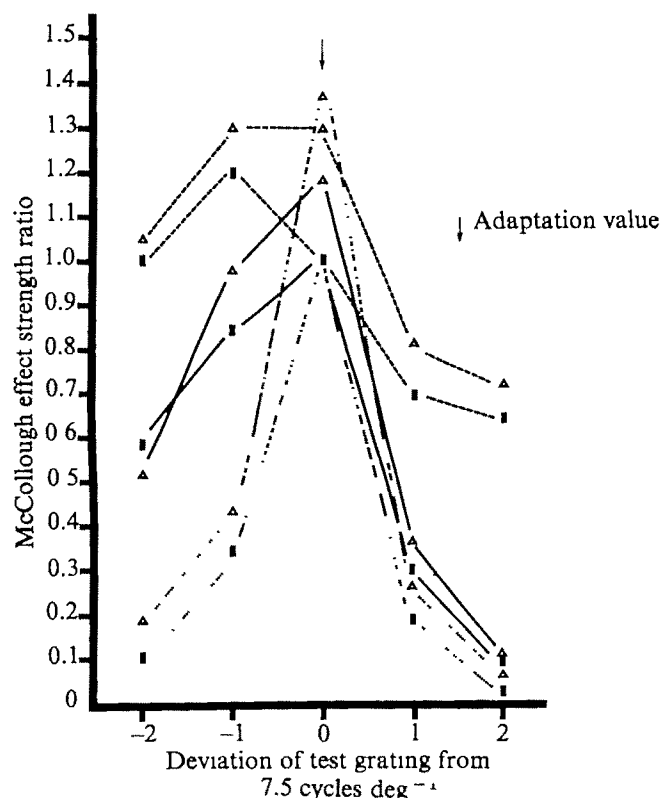
mission between the hemispheres to appropriate processing centres, (2) greater attentional activation of one hemisphere depending on task requirements and (3) the right hemisphere superiority in visual perception is due to sensitivity on processing differences possibly down to area 17 (ref. 21).

The first theory implies that the rating procedure is a function of the right hemisphere and that information from the left is degraded as it is transferred to right for judgement. One might predict, however, that such a process would increase the variability of the left hemisphere scores but no significant trend for larger left hemisphere variances were found for any subject (Bartlett-Box F , $P > 0.10$). However, a constant decrement is possible and cannot be totally eliminated.

The second model²² assumes that if a cognitive strategy proper to one cortical hemisphere is adopted, attention is biased to the contralateral visual fields. If hues ratings activated the right hemisphere to a greater extent, this would explain the present data. Attentional biases in this model, however, are generated in favour of the left hemisphere if there was an expectancy of a verbal processing which the present experiment did include. Thus, the present data are contrary to the original model. Also, experiments designed to reduce any attentional biases still demonstrate hemispherical effects²¹.

The superiority of the right hemisphere in visual tasks, as postulated by the third theory, might be due to enhanced contrast of the stimulus or a longer duration of the stimulus trace⁹. Either one of these postulated improvements in visibility operating on the adaptation and test stimuli could produce stronger McCollough effect hues. Also, if the

Fig. 3 McCollough effect strength ratios for subjects JC, WH and GL as test grating spatial frequency deviated from 7.5 cycles deg^{-1} of visual angle (0 octaves) as a function of cortical hemisphere of initial stimulus presentation. The adaptation frequency is indicated with an arrow. Each point represents 60 ratings. \triangle — \triangle : JC, right hemisphere; \blacksquare — \blacksquare : JC, left hemisphere; \triangle — \triangle : WH, right hemisphere; \blacksquare — \blacksquare : WH, left hemisphere; \triangle — \triangle : GL, right hemisphere; \blacksquare — \blacksquare : GL, left hemisphere.



orientation specific adaptation phenomenon with its hypothesised loss in sensitivity in cortical units is a neural modification akin to memory, it might be more efficiently and effectively implemented for perceptual tasks in the right hemisphere given the latter's predominance in such processing.

Information transmission problems and attentional shifts are possible but the variation of early cortical sensitivity seems to be the more parsimonious and interesting potentiality at this stage of analysis. If the latter is true, whether these differences are developmental in nature or innate is quite fascinating. In primates, the striate populations are stimulus selective at birth but modifiable by experience²⁰. Any interaction between visual cortical perceptual development and the laterality of information processing is relatively unexplored.

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Effect of monocular deprivation on binocular neurones in the owl's visual Wulst

DESPITE the two hundred million years during which their evolutionary history was different from mammals, birds possess a central visual apparatus with surprising functional similarities to the striate cortex of cats and monkeys¹. The visual Wulst² of the owl contains neurones which can be binocularly activated and which show precise selectivity for the orientation, direction of movement and binocular disparity of moving straight line contours¹, all characteristic properties of single neurones recorded from the striate cortex of cats and monkeys^{3,4}. We were interested to determine whether binocular neurones in the owl's Wulst are also sensitive to visual experience in the neonatal period since another important characteristic of binocular neural connections in cat and monkey visual cortex is their extreme sensitivity to monocular deprivation during the critical period^{5,6}. The preliminary observations we present here, on young, monocularly-deprived owls, suggest that the functional parallel between the mammalian striate cortex and the avian Wulst extends to the phenomenon of plasticity as well.

Data were obtained from 8 barn owls (*Tyto alba*) which had been hand-raised indoors. Six had relatively normal early visual experience in the laboratory and provided control data for this study in addition to detailed data presented elsewhere¹. The ages of the 6 normal owls ranged from 1 to 7 years. Two owls had had the right eyelid sutured under halothane anaesthesia (1-1.5% in 50% O₂: 50% N₂O) at the ages of 11 d, at the time of normal eye opening (Owl T) and 29 d (Owl D, both of whose eyes had opened at 7 d) respectively. Both monocularly lid-sutured owlets seemed unaffected by the procedure if we compared their behavioural development with normal owlets. For example, the typical, side-by-side, scanning head movements were first used by Owls D and T to locate visual targets at the same age as normal owlets.

Single unit recording was carried out with tungsten-in-glass extracellular microelectrodes⁷ from a closed chamber over the lateral margin of the visual Wulst (representing the visual field within 10° of the vertical meridian¹) under ketamine anaesthesia (12 mg/kg intramuscular).

All birds, including the two monocularly-deprived owls, were fully grown (average 550 g) and fully fledged at the time of recording. Owls T and D were 3 and 4 months old, respectively. Corneas were protected with plastic contact lenses (radius of curvature, 6.5 mm). Visual stimuli were presented on a rear projection screen 57 cm from the nodal point of the eye by a custom-built projector which allowed joystick control of position, orientation and size^{8,9}. Retinal landmarks were projected ophthalmoscopically on to the screen and the vertical meridian and visual axes established indirectly, as is done in the cat¹⁰, from the position of the optic nerve head, which is overlaid in birds by the easily distinguished pecten oculi¹. Variable biprisms set before each eye made it possible to control binocular retinal disparity¹.

As described in detail elsewhere¹, the Wulst of the normal owls contained a high proportion of binocularly activated neurones, including a significant fraction which were driven only by simultaneous binocular stimulation with targets at the appropriate binocular disparity (Fig. 1). These latter neurones, which show functional similarities to the binocular depth cells recorded in the extrastriate visual cortex of monkey¹¹, were found only in the more superficial layers of the Wulst, around 1 mm from the surface.

In marked contrast, both monocularly deprived owls lacked binocularly activated neurones (Fig. 1). In the superficial layers, where we encountered the cells with an absolute requirement for binocular stimulation in normal owls, many cells had spontaneous activity but were unresponsive to any visual stimulation we could devise. In the deeper layers where we recorded the majority of neurones, there were uninterrupted sequences of cells with brisk responsiveness and receptive fields which could be sharply defined with moving edges of the appropriate orientation and direction of movement. These sequences were therefore like those through the Wulst of normal owls, except that the receptive fields could be defined only for one eye, which was in most cases the non-deprived eye. We also recorded a number of neurones, particularly from Owl T, which had receptive fields in the deprived eye only. All of these cells had poorly defined preferences for stimulus orientation but were briskly responsive, a strong indication that there was a functional input to the Wulst from the deprived eye.

Another striking difference between normal and monocularly-deprived owls was the presence of a marked strabismus in the latter. As measured by the projection of the tip of the inferior limb of the pecten oculi, where it meets the optic nerve head, eye alignment is extraordinarily precise in normal owls. The pecten tips of each eye are in the same horizontal plane and are separated by 56° (s.d. ± 0.9°, N=6) when projected into space through the nodal points.

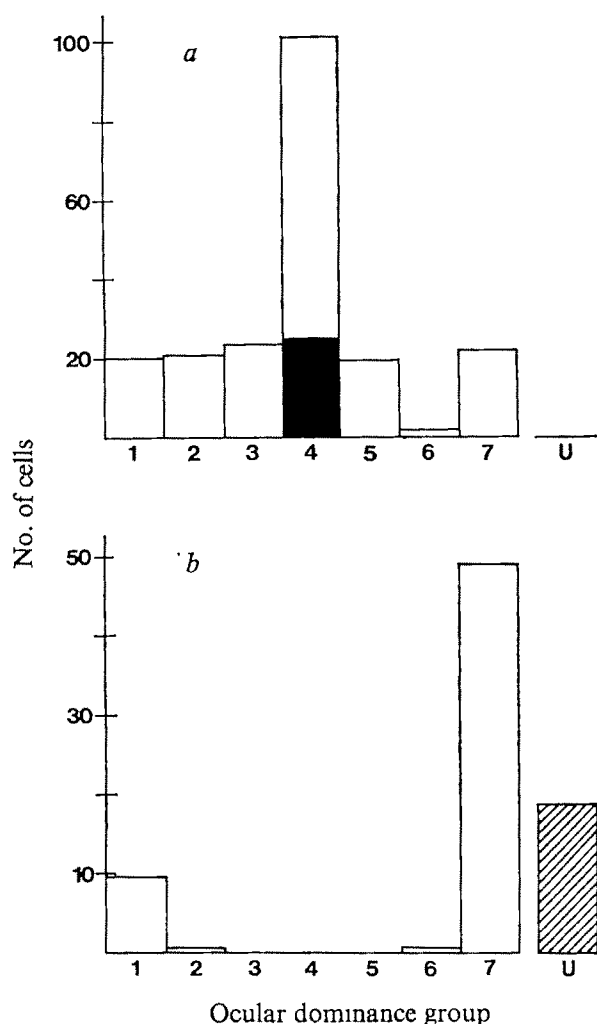


Fig. 1 Ocular dominance histograms obtained from the visual Wulst of 6 normal owls (a) and two owlets each of whose right eye had been closed for 2–3 months (b). All data shown were obtained from electrode tracks in the left hemisphere. A total of 10 electrode tracks provided the data in a and two tracks (one in each of the monocularly deprived owls, T and D) provided the data in b. A third electrode track in the right hemisphere of Owl T yielded seven visually unresponsive cells and seventeen driven exclusively by the contralateral (non-deprived) eye. These data are not included in the figure to avoid possible confusion caused by the reverse order of ocular dominance numbers which would apply to the opposite hemisphere. Single neurones were assigned an ocular dominance category according to the criterion of Hubel and Wiesel³, where group 1 represents cells driven exclusively by the contralateral eye, group 4, cells driven equally well by both eyes, and group 7, cells driven exclusively by the ipsilateral eye. The group 4 cells in the filled portion of histogram a could be driven only by simultaneous presentation of an oriented target to both eyes at a certain binocular disparity and could not be driven independently from either eye. The shaded area of histogram b, marked "U" indicates cells which were unresponsive to visual stimulation. Both the filled cells in a and the shaded cells in b were recorded in the superficial layers of the Wulst.

There was clear vertical and horizontal misalignment in both owls T and D whose pecten tips were separated by 66° and 96° respectively. Although strabismus is a feature of monocularly deprived kittens¹² it was very surprising to us in this case because of the absence of eye movements greater than a degree or two in adult owls^{1,13}. Perhaps eye movements are more prominent in the owl's early development when they could allow the final precise alignment to be achieved with visual feedback.

The present findings strongly suggest that binocular visual experience is as important for the development of the avian visual Wulst as it is for mammalian visual cortex.

We postulate that a requirement for binocular input during development may be a universal feature of binocular visual systems which have evolved to subserve the subtle disparity-detection task required for stereopsis⁴. We do not yet know how age dependent are the effects of monocular deprivation in the owl, nor do we know whether binocular neurones can be found in the Wulst before visual experience of any kind.

Finally, it should be pointed out that the striking functional similarities between visual Wulst and visual cortex exist in spite of a number of differences between their respective visual pathways. These organisational differences might be exploited for a better understanding of both systems. For example, since the input and output fibre pathways of the Wulst are separate, one could test the role played in monocular deprivation by the output pathway from the Wulst back to its thalamic input, an impossible experiment in mammals, where both input and output fibre pathways run together.

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Monocular deprivation in kittens impairs the spatial resolution of geniculate neurones

SELECTIVE exposure of kittens to periodic gratings during the early period of life produces a depression of neural responses to gratings of the same or similar spatial frequency¹. Recently we have found that in these kittens also the behavioural contrast sensitivity is depressed at the spatial frequency of exposure (A.F. and L.M., in preparation).

Plastic changes in the functional properties of the visual system caused by restricted visual experience during the critical period have been described mainly at cortical level, although for instance total deprivation of visual experience in one eye can induce anatomical changes in the lateral geniculate (LGN) layers². The early exposure to periodic gratings, however, is found to affect the spatial frequency characteristics not only at cortical level but also at the level of the LGN¹. This suggested to us that deprivation of visual stimuli might also effect the spatial frequency responses of geniculate neurones. We report here evidence suggesting that monocular deprivation impairs the resolution of the neurones in the corresponding layers of the geniculate body. A similar result has recently been obtained by Ikeda and Wright³ who have reported that in strabismic kittens the resolution of geniculate cells is impaired.

Experiments were performed in 4 kittens in the first week of their life. Using halothane anaesthesia we sutured the

eyelids of one eye (in 3 kittens the right eye, in one kitten the left eye). In addition to suturing the eyelids we drew the nictitating membrane across the cornea and sutured it to the conjunctive along the upper lid.

The recording experiments were performed when the kittens were at least 3 months old. On the day of the experiment the cat was anaesthetised with halothane, and endotracheally intubated. Two small openings were made over the projections either of the lateral geniculate bodies or of the optic tracts. Afterwards the animal was immobilised with curare and anaesthesia was continued with nitrous oxide (80% NO_2 + 20% O_2). Animals' pupils were dilated with atropine, contact lenses with artificial pupils 4 mm in diameter were applied to both eyes and refraction was carefully corrected with additional lenses.

The action potentials of geniculate neurones or optic tract fibres were recorded extracellularly with tungsten microelectrodes and receptive fields were mapped by projecting the images of bright spots or bars on to a tangent screen 57 cm from the cat's eye. For each isolated neurone we obtained the average responses to 20 stimulus repetitions using drifting square-wave gratings of high contrast and various spatial frequencies (for details of the method see ref. 4).

We have done 9 penetrations in the LGN and one in the optic tract recording from 121 neurones. For each neurone we measured the "visual acuity", defined here as the highest spatial frequency of the drifting grating that produced a just detectable modulation of the rate of discharge in the averaged responses. The receptive fields of the neurones recorded in each penetration were at least partially superimposed and fell within a restricted area of the visual field. In some penetrations this area was within the area centralis, in others it was eccentric to it, but never more than about 20° . We have found that the average visual acuity of neurones activated by the deprived eye is approximately one half that of the neurones activated by the normal eye and projecting to the same part of the visual field. In Fig. 1 we present the visual acuity of neurones for a penetration through the LGN ipsilateral to the deprived eye (a) and for a penetration through the LGN contralateral to the deprived eye (b). It is clear that going from a layer to the next (from filled circles to open circles, or *vice versa*) brings about an abrupt change in the average visual acuity.

The results obtained in all the penetrations are summarised in Table 1. For each penetration, we reported the number, N , the mean visual acuity, m , and the standard

Table 1 Acuity of cells in normal and deprived eyes of monocularly-deprived kittens

Penetration	Cat	Normal eye			Deprived eye		
		N	m	s.d.	N	m	s.d.
1	1	9	2.1	0.4	8	0.9	0.3
2		4	2.0	0.4	7	0.8	0.3
3	2	4	2.1	0.8	8	1.1	0.9
4		4	1.7	0.4	6	0.5	0.3
5		9	2.0	0.5	11	0.8	0.3
6		7	1.9	0.6	6	0.8	0.2
7	3	4	3.1	0.2	5	2.8	1.1
8		8	1.5	0.3	10	0.8	0.3

deviation of cells activated by the normal eye (left columns) or by the deprived eye (right columns) and located in either of the two upper layers of the LGN (layer A, contralateral eye, and A_1 , ipsilateral eye). Penetrations 1, 5 and 6 were in the LGN ipsilateral to the deprived eye, penetrations 2, 3, 4 and 7 on the opposite side. All the cells recorded had receptive fields located within 5° from the centre of the field, except for penetration 4, that projected 10° peripherally. Two penetrations in which we recorded only 4 and 6 neurones (kitten 2 and 4, respectively) have not been included in Table 1. A few neurones located in layer C have also been omitted. In the results we have reported we have not discriminated between X and Y neurones, because we found that the great majority of cells recorded in the deprived layer had lower visual acuity than the neurones in the normal layer.

The data for penetration 8 in Table 1 were obtained recording from optic tract fibres projecting to a restricted area of the visual field. They look very much like the statistics evaluated for similar samples of neurones of the LGN, suggesting that the retinal neurones of the deprived eye might be involved in the loss of visual acuity observed at the LGN level. A similar hypothesis was proposed previously¹. Further recordings from retinal fibres would be required to assess this point.

We conclude that visual deprivation of one eye during the early period of life in cat impairs, on the average, the spatial resolution of the neurones in the LGN on to which the deprived eye projects.

Experiments are in progress to investigate whether the plasticity observed in LGN neurones is of cortical origin or is present even at a retinal level, as the one penetration we did in the optic tract could indicate.

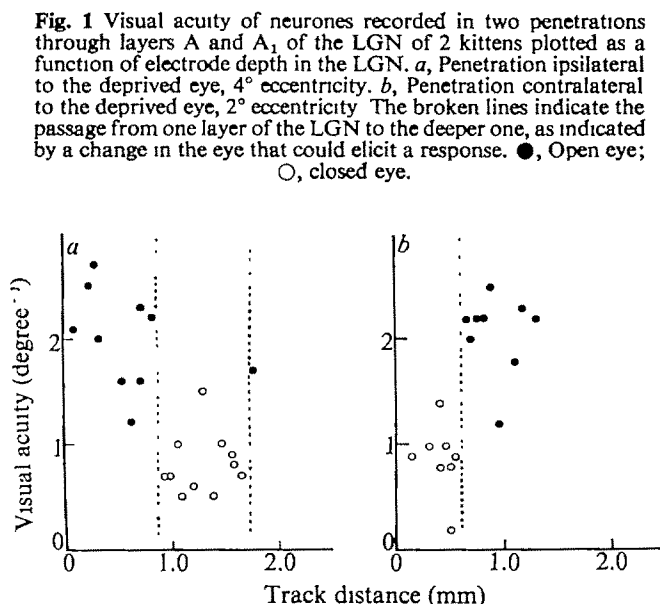
Ikeda and Wright² suggested that the loss of resolution in LGN neurones of cats with surgically induced squint could have a bearing on the human strabismic amblyopia. The similar effect induced in our kittens by deprivation from patterned vision could be at the basis of the amblyopia caused in human subjects by anisometropia (when patterned vision is dramatically impaired in one eye by a large optical defect) or by corneal or lens opacities occurring in one eye in the early years of life. The fact that neural resolution can be affected in the deprived animals is a further warning against the prolonged patching of one eye, a practice still in use in the treatment of squint.

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Uptake of soil capillary water by ghost crabs

SEVERAL semiterrestrial crabs, including some ghost crabs (genus *Ocypode*) can extract interstitial water from damp sand¹⁻³. This enables them to offset their high evaporative losses even in habitats lacking surface water. The mechanism involved has remained unknown, although there have been a few observations of how *Gecarcinus lateralis* takes up droplets of water applied to its venter⁴. I have found that uptake of soil water by *Ocypode quadrata*, the common ghost crab of Caribbean and temperate Atlantic North American beaches, is accomplished by a mechanism involving capillary attraction and the production of a substantial vacuum.

Uptake of soil water by terrestrial arthropods requires at least two steps. First, water must be collected by a force exceeding the capillary attraction of the soil spaces. Second, the water must be drawn, past the cuticular exterior, into contact with an ion- and water-transporting epithelium for absorption. These steps have been elucidated only in some small spiders, which can draw up water directly from soil spaces with the sucking mouthparts, against capillary attractions of up to 500 mmHg, and transfer it to the gut for absorption⁵.

In semiterrestrial crabs, which are much larger, the water-collection step has been attributed to tufts of setae located on the bottom parts of the body^{1-3,6}. In *Ocypode quadrata* prominent tufts occupy the bases of the second and third walking legs. The tufts (shown spread apart in Fig. 1) are usually closely apposed and enclose the single posterior aperture to each branchial chamber.

Several experiments indicated that these hydrophilic tufts were essential to the uptake process. Crabs desiccated to 5-10% weight loss, when placed on damp sand, promptly sat down and brought the tufts into intimate contact with subsurface sand by oscillating briefly from side to side. The tufts soon became saturated with water. Water collection, and thus uptake, was prevented if contact of the tufts with sand was prevented by gluing on rubber flaps as a loose cover, or if the capillarity of the tufts was destroyed by matting them with lacquer.

Although the tufts account for water collection, they do not seem to be the site of absorption into the blood. The tufts require cuticular stiffening to penetrate the sand; thick cuticle usually prevents rapid water or ion transport. Also, crabs on sufficiently damp sand bubbled surplus water out around the mouthparts, suggesting that water was transferred to the branchial chambers. When desiccated crabs were placed on sand dampened with seawater containing 0.1% of methylene blue, the tufts did not stain darkly. Within 30 min, however, the gills stained intensely, and within 60 min the mouthparts were moderately stained by the dye solution bubbling out around them. *Ocypode* gills⁷, and portions of *Gecarcinus* gills⁸, have fine structure typical of osmoregulatory rather than gas-exchange membranes. These observations indicate that the absorption step is the function of the gills and that the tufts accomplish only water collection.

The gills and tufts are some distance apart (Fig. 1), and so water must be transferred from the area of collection to the area of absorption by a force exceeding the capillary attraction of the tufts, which itself must exceed the capillary attraction of the soil. Although capillary channels under the carapace can apparently conduct free water to the gills of *Gecarcinus*^{4,9}, it seems unlikely that they could exert the force required to extract soil water. In any case, no such channels communicate with the tufts of *O. quadrata*, and capillarity cannot account for the movement of water to the gills through its relatively large posterior passages. The most

obvious alternative hypothesis, that some sort of wringer action squeezed the water out of the tufts, was eliminated because water could move from tufts to gills without tuft or leg movement.

The remaining hypothesis, that the crabs could generate sufficient vacuum to suck the water from the tufts into the gill chamber, seemed improbable. To test this hypothesis, I drilled through the calcified carapace over each branchial chamber of several crabs (under CO₂ anaesthesia), but not through the underlying membrane, with a high-speed diamond-abrasive dental drill. The highly vascular chamber lining was perforated with a red-hot wire to cauterise it and minimise the otherwise copious bleeding. Cannulae (cutoff 20-gauge hypodermic needles) were fixed with epoxy resin into the holes so that the branchial chambers could be vented or sealed easily. Desiccated crabs with open cannulae were unable to draw up water from damp paper towelling, or even from a thin film of free water. Fourteen such animals lost an average of 0.01% body weight during 8 h on damp sand. Within 4 h of the cannulae being sealed, however, they had gained an average of 6.8% body weight on the same sand, indicating that venting, not surgical trauma, prevented water uptake.

To verify that the uptake of water when cannulae were sealed was indeed due to negative pressures produced in the branchial chambers, I connected the cannulae of three desiccated crabs to a Physiograph recorder equipped with pressure transducers (E & M Instruments). When the crabs were placed on damp sand (5.0-7.5% distilled water) they immediately worked their tufts into the sand. After several minutes, during which the tufts became wetted by a continuous water film, the branchial chamber pressure abruptly fell from ambient to as much as 40 mmHg below ambient, where it remained for a few seconds to several minutes. This was repeated at irregular intervals (Fig. 2).

The maximum suction produced was surprisingly high: in each crab tested it was sufficient to draw up a column of water 55 cm high. This is particularly remarkable in view of the constraints of crustacean structure. The fluttering of the scaphognathite of the first maxilla in a close fitting passageway is adequate to move water through the branchial chambers of aquatic crabs. The movement of air against a large pressure gradient, however, requires much better

Fig. 1 *Ocypode quadrata* with portion of carapace cut away, showing relative positions of structures involved in water uptake. Surplus water may be dumped from the branchial chamber, around the mouthparts, through a passage (not shown) under the antero-lateral carapace

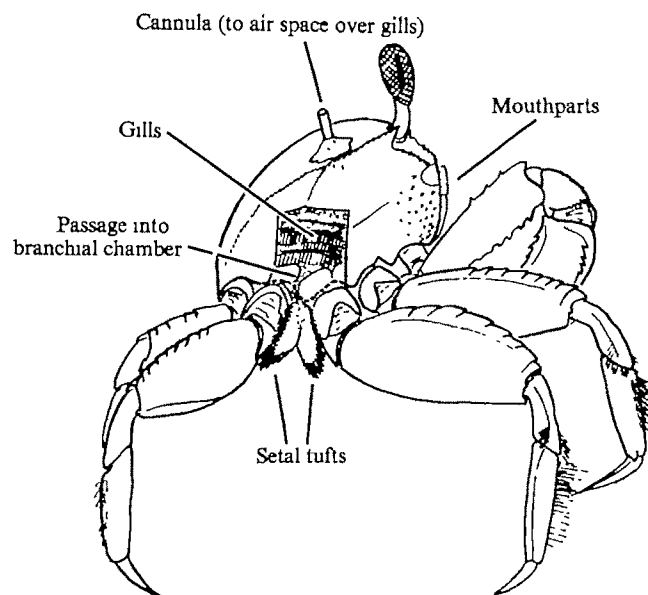
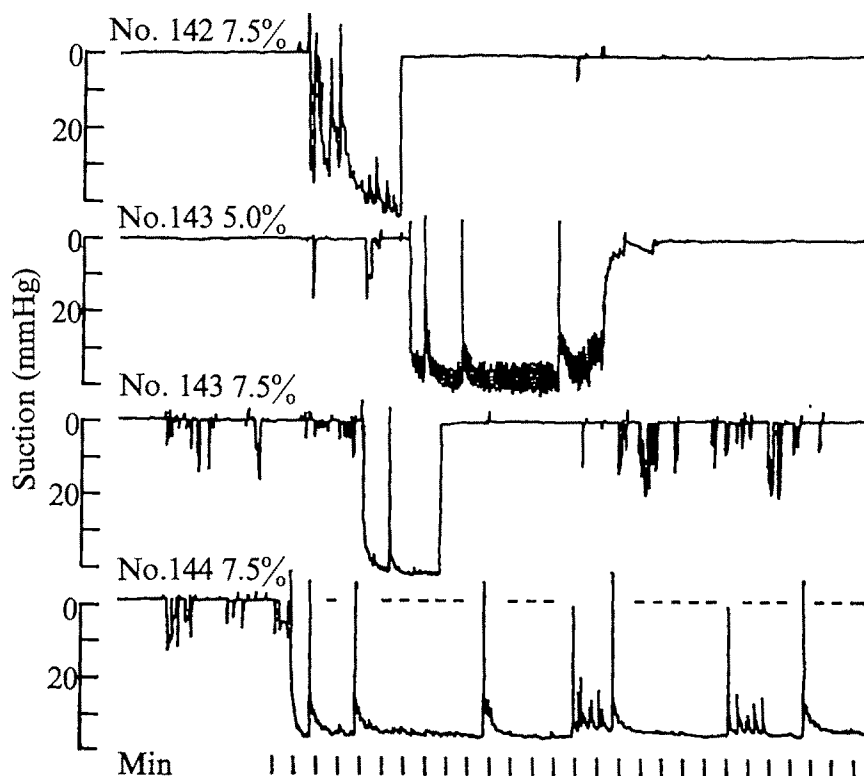


Fig. 2 Representative segments of pressure recordings from branchial chambers of desiccated crabs on damp sand (5–7.5% distilled water). Traced from original recordings.



sealing and the addition of one-way valves to such a pump. The scaphognathite is evidently used in vacuum production by *O. quadrata*, but how it and associated structures have been modified for this purpose is not clear. Also remarkable is the airtight seal formed at the carapace margin, even though fusion of the margin with the irregular area where the legs join the body is precluded by the necessity of shedding the branchial chamber lining at moulting. The seal seems to consist of a close set row of fine setae fringing the carapace margin. These presumably retain a film of water by strong capillarity; the water serves as the gasket.

At the threshold soil-water content for uptake by *O. quadrata* (3–5%), maximum suction produced by the crabs exceeds soil capillarity (the soil–water tension commonly measured by soil scientists) by 2–10 mmHg. The excess suction represents the pressure gradient available to move water from soil spaces through the tufts to the gills. It is interesting that as soil water content drops below 3–5%, capillary attraction, and thus the vacuum required to remove additional water, increase considerably. *Ocypode quadrata* has evidently refined its vacuum mechanism to the limits of practicality.

The water collection and vacuum mechanisms involve only bulk transport of water; consequently their operation is identical whether sand is dampened with distilled water or seawater. The crabs, however, do not take up fluids indiscriminately, but avoid those likely to cause osmotic stress. For example, desiccated crabs given sand dampened with distilled water rapidly regain weight up to their approximate initial weight and then avoid further uptake; crabs given seawater-dampened sand continue to collect water and bubble the surplus from the branchial chamber.

The presence of setal tufts associated with water uptake in several genera of semiterrestrial crabs (*Gecarcinus*^{1,2}, *Cardisoma*³, *Ocypode*^{2,4}, *Sesarma* (personal communication from L. Abele), and *Uca* (personal communication from L. Powers)) probably represents convergent evolution stemming from a shared problem. The setal tufts may have served as intake screens during the intertidal phase of terrestrial invasion, preventing fouling of the gills by suspended sediment particles. To exclude particles while passing respiratory water, the setae would have to be closely

spaced, stiff and wettable—attributes which would preadapt them for soil water collection. Well sealed branchial chambers and air pumps may also be regarded as adaptations to intertidal life, facilitating particle exclusion and permitting survival during tidal exposure.

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Colony incompatibility in bacteria

WE report here the demonstration of colony incompatibility between different strains of the same species of various Enterobacteriaceae, using chemotactic migration through semi-solid agar. This phenomenon has hitherto only been demonstrated in the swarming bacteria, *Proteus*, where it is known as the Dienes phenomenon.

Proteus bacteria are unusual in that they readily swarm out from the inoculum point on the surface of most agar media. Despite study by many workers there are several aspects of this swarming that are not understood. We have been considering one of these aspects, namely the behaviour of two swarms as they approach one another. When two different swarming strains of *Proteus* meet on agar there often occurs what has been described as “one of the most peculiar forms that antagonism can assume”. This effect, known as the Dienes phenomenon, as well as the swarming of *Proteus*, has been illustrated in a review¹. Some hours after the two swarming colonies have met and merged, a

demarcation zone about 1 mm wide of sparse bacterial growth and containing many aberrant cells appears^{3,4}. The effect has been of some value in epidemiological studies^{5,6} in determining whether two isolates belong to different strains. Although 30 years have elapsed since the phenomenon was discovered⁷, little progress has been made towards elucidating its basis, nor have comparable forms of colony incompatibility been reported in other bacteria.

Is the phenomenon therefore the result of a form of incompatibility occurring only in *Proteus*, or is it an unusual expression of a more widespread antagonism (or of antagonisms), the unique features being associated with the spectacular swarming characteristic of *Proteus*? Swarming and the Dienes phenomenon are usually demonstrated on a firm agar medium, and on such media other bacteria do not swarm. Bacteria can, however, be induced to migrate rapidly by means of chemotaxis, an effect widespread in microorganisms⁸. Adler⁹ showed that with soft agar media¹⁰ a form of swarming—chemotactic migration through agar—can be produced in *Escherichia coli*. We have now used this soft agar medium to demonstrate colony incompatibility in *Proteus mirabilis*, *E. coli*, and *Salmonella* spp.

Replicated tests were carried out at 37 °C in a water-saturated incubator on four strains of *Proteus mirabilis* (2953, 2960, CW 237 and CW 349, obtained from Mr T. L. Pitt, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT) on firm and soft nutrient agar (Oxoid Nutrient Broth, 1.3%; Difco Bacto-Agar at 1.5% or 0.35%) and firm and soft Tryptone agar (Difco Bacto-Tryptone, 1%; NaCl, 0.5%; Difco Bacto-Agar at 1.5% or 0.35%) in all possible combinations of pairs. Petri dishes were left 2–3 d before use to ensure that the agar was free of drops of surface water which would cause spreading rather than swarming of colonies. On both the firm agar media the colonies spread rapidly across the agar surface, with the occasional pauses of about an hour characteristic of zoned swarming. Inoculation of one strain at one side of the Petri dish and the other strain at the opposite produced, in all but one combination, a clear Dienes line where the swarming colonies met at the centre of the dish. With Petri dish lids removed, under good illumination and against a dark background the demarcation line could be recognised as a zone of less dense growth, and viewed obliquely, one or two ridges could be seen at the surface representing sharp limits to dense growth. Although Dienes reactions were usually obtained within 1 d, the surface ridges often became more pronounced later, so recording was carried out at 2–3 d. Control cultures in which a single strain was inoculated at opposite sides of the Petri dishes showed neither diminished growth nor surface markings where the colonies had met. On both the soft agar media the bacteria migrated from the point of inoculation through the medium as circular bands of steadily increasing radius, as described for *E. coli* by Adler⁹, with subsequent dense growth behind the advancing front. Where two colonies of an identical strain meet at the centre of a Petri dish there is often an area of more sparse bacterial development than elsewhere—possibly the simultaneous arrival of two bands of bacteria leads to rapid nutrient depletion and chemotactic migration out of the area. In consequence of this behaviour in controls, sparse growth where colonies meet is not a reliable indication of incompatibility of soft agar media, and assessment has to be on the basis of surface ridges alone. By this criterion, two combinations of strains (CW 237×CW 349, CM 237×2953) showed clear incompatibility on both firm and soft agar media, three combinations (CW 237×2960, 2960×CW 349, 2960×2953) on firm but not on soft agar, and one combination (CW 349×2953) on soft agar only. Hence all combinations of strains displayed incompatibility, but in some instances it was more readily demonstrated on firm agar and in some with soft.

Tests were carried out with several strains of *E. coli*

and *Salmonella* spp. on soft Tryptone agar. Before experiments strains were passaged through Cragie tubes to ensure motility¹¹. Migration, growth and incompatibility reactions similar to those of *Proteus* on soft agar media were obtained with both *E. coli* and *Salmonella* spp. Some pairs of strains consistently gave reactions and others consistently did not. With *E. coli*, for example, NCTC (National Collection of Type Cultures) 9002 never gave a reaction with NCTC 9001, but both gave reactions with NCTC 9004. It is concluded that colony incompatibility reactions resembling the Dienes effect occur in members of the Enterobacteriaceae other than *Proteus*, and that the previous recognition of the phenomenon in *Proteus* alone was a consequence of the striking form the reaction takes in this genus which is capable of fast swarming on firm agar.

One of us (K.A.B.) intends to investigate the ecological and pathological implications of colony incompatibility in *E. coli* and *Salmonella*, the other (M.J.C.) the ultrastructural, genetic and biochemical basis of the effect in *E. coli* and *Proteus*. It is possible that bacteriophage or bacteriocins are responsible, even though in some instances claims to have excluded one of other of these possibilities have been made¹; on the other hand, some novel form of antagonism may be involved.

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Suppression of malaria infection by oxidant-sensitive host erythrocytes

DEFICIENCY of the red cell enzyme, glucose-6-phosphate dehydrogenase (G-6-PD), predisposes erythrocytes to oxidant-induced haemolysis and is thought to protect humans against severe malaria infection. We have already shown that the malaria parasite exerts an oxidant stress on infected red cells and suggested that premature lysis of malaria infected erythrocytes might occur in G-6-PD deficient humans¹, thus limiting the severity of infection by enforcing the release of immature parasites incapable of propagating the infection².

The geographical coincidence of several types of quantitative deficiency of G-6-PD and endemic malaria suggests that these X-linked enzyme deficiencies have an adaptive advantage in malaria-ridden human populations. Although experimental inoculations of G-6-PD deficient human volunteers with *P. falciparum* have not revealed any difference in the early course of the disease³, both epidemiological^{4,5} and direct⁶ evidence from naturally occurring infections indicates that this enzyme deficiency does protect against fulminant malaria. Vitamin E deficiency confers an oxidant

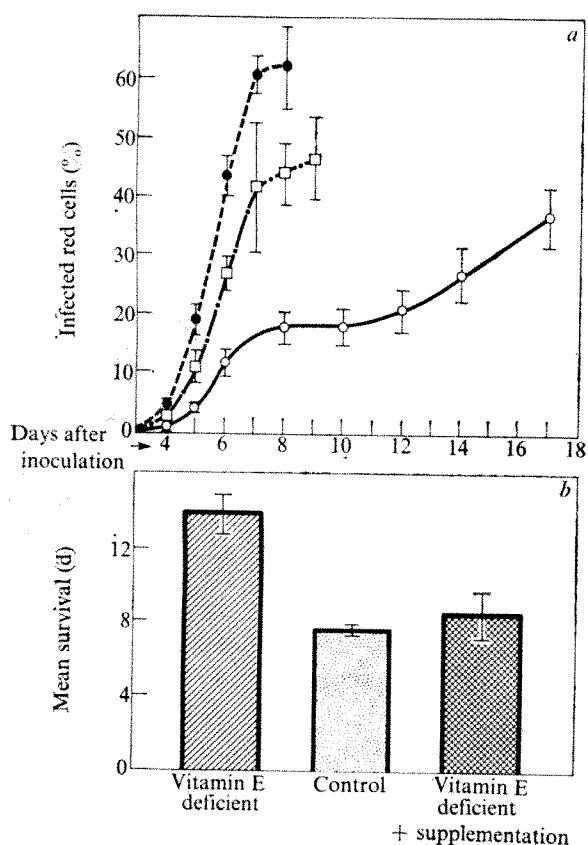


Fig. 1 *a*, Percentage infected red cells in 19 mice fed normal laboratory chow (●--●), 21 mice fed a vitamin E-deficient diet for 60–100 d (○—○), and 8 mice fed a vitamin E-deficient diet for 60–100 d and then resupplemented with vitamin E for 30 d (□--□). Resupplementation of the latter group ended 2 d before inoculation. Each point represents the mean parasite count and the vertical bars through the points represent ± 1 standard error of the mean (s.e.m.). *b*, Mean survival of 19 control, 21 vitamin E deficient and 8 vitamin E-deficient resupplemented mice after inoculation with *P. berghei*. Vertical bars represent ± 1 s.e.m.

sensitivity on the erythrocyte which is similar in many ways to that in G-6-PD deficiency. We now report that vitamin E deficiency moderates the course of *Plasmodium berghei* infection in mice, thus supporting the idea that oxidant sensitivity of host erythrocytes—whether due to G-6-PD or vitamin E deficiency—may limit the severity of malaria.

We recently found that in *P. berghei*-infected mice (1) there is an increase in steady-state methaemoglobin concentration which is directly related to the severity of infection, (2) catalase within infected erythrocytes is extensively inhibited by 3-amino-1,2,4-triazole (an inhibition which requires the presence of H_2O_2) and (3) infected erythrocytes accumulate 10–20 times as much methaemoglobin as do normal red cells when exposed to oxidants generated by ascorbic acid¹. These malaria-infected erythrocytes are thus subject to internally generated oxidants and are also abnormally sensitive to exogenous oxidant stress.

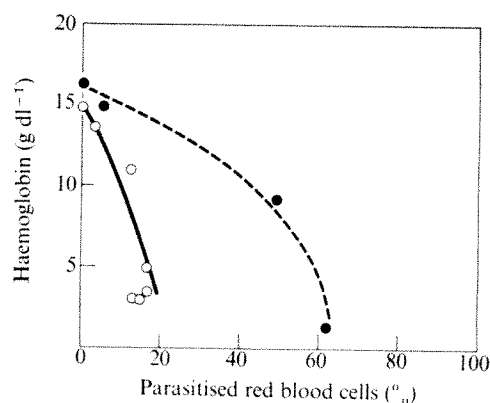
We have now undertaken experiments to determine whether the severity of murine *P. berghei* infection might be diminished by oxidant sensitive host erythrocytes induced by feeding mice a vitamin E-deficient diet. Vitamin E deficiency was used as a model for three main reasons. First, vitamin E normally protects erythrocyte membranes against oxidants and vitamin E deficiency predisposes red cells to peroxide-induced haemolysis^{7–9}. Second, during the earlier experiments we observed that plasma vitamin E concentrations in *P. berghei*-infected mice were approximately half those in uninfected animals, suggesting that oxidants of parasitic origin might increase vitamin E consumption [plasma tocopherol concentrations in 8 uninfected mice =

0.33 ± 0.013 mg per 100 ml and in 14 infected mice 4 d after inoculation = 0.17 ± 0.013 mg per 100 ml; $t = 8.03$, $P < 0.001$ (Student's *t* test, two-tailed)]. Lastly, recent evidence from some areas of endemic malaria suggested that malnourished African herding peoples, whose diet is normally low in vitamin E, are relatively resistant to severe malaria infection. Feeding these people cereal grains (an excellent source of vitamin E) causes recrudescence of previously undetectable *P. falciparum* infections¹⁰.

Swiss white mice were fed either regular laboratory chow or a vitamin E-deficient diet (Nutritional Biochemicals, Inc.) for 60–100 d. Vitamin E resupplementation of animals previously made E deficient was accomplished by the intraperitoneal injection, every 48 h, of a solution of α -tocopherol (100 mg kg^{-1} body weight) in propylene glycol and absolute ethanol¹¹. In mice on the vitamin E-deficient diet, vitamin E levels reached a nadir after 50 d, as assayed by the peroxide haemolysis test¹² and analysis of plasma tocopherol¹³ [control = 0.34 ± 0.03 mg per 100 ml ($n = 16$), vitamin E deficient = < 0.10 mg per 100 ml ($n = 9$)]. The animals were then inoculated intraperitoneally with 2×10^6 red cells infected with *P. berghei* (NYU-2 strain) as previously described¹. Blood was periodically obtained by incision of the caudal veins, and the percentage parasitised erythrocytes was determined by light microscopic examination of a minimum of 500 cells.

Vitamin E-deficient mice are more resistant to *P. berghei* as reflected by slower development of parasitaemia (Fig. 1*a*) and much longer survival (Fig. 1*b*). This resistance seems to be due to an inability of the mature vitamin E-deficient erythrocyte to support the parasite; almost all infected red cells in the deficient mice are reticulocytes whereas in control animals the parasite is found in almost equal frequency in reticulocytes and mature red cells (the ratios of percentage infected reticulocytes to percentage infected mature erythrocytes 7 d after inoculation were: controls, 1:1 and vitamin E deficient, 9:1). Furthermore, deficient mice become more anaemic in proportion to the severity of infection than do normal controls (Fig. 2). The reticulocyte preference of the parasite as well as the severe anaemia in vitamin E-deficient animals suggests that erythrocytes are being infected with normal frequency, but are destroyed abnormally rapidly. It seems that only metabolically competent reticulocytes are able to support full maturation of the parasite. This is consistent with earlier work¹⁴ showing that reticulocytes of vitamin E-deficient rats are resistant to haemolysis induced by exposure to high oxygen pressures *in vivo* and to peroxide *in vitro*. Thirty-day resupplementation of mice still being fed the vitamin E-deficient diet almost fully restores their susceptibility to

Fig. 2 Decline in whole blood haemoglobin concentration of 19 control (●) and 21 vitamin E-deficient (○) mice after infection with *P. berghei*. The points represent the mean haemoglobin and mean percentage infected red cells for all animals.



P. berghei (Fig. 1a and 1b). Furthermore, in these resupplemented animals, the parasite loses its tendency to inhabit only reticulocytes (the ratio of % infected reticulocytes to % infected mature red cells in vitamin E deficient resupplemented mice was 2:1). The diminished susceptibility of vitamin E-deficient mice to malaria infection, therefore, seems to be a specific function of vitamin E-deficiency and not of other deficiencies incurred as a result of feeding the artificial vitamin E-deficient diet.

Quantitative deficiency of G-6-PD is also thought to supply at least a modicum of protection against severe malaria infection. Not only are high frequencies of this enzyme deficiency found in human populations with a history of endemic malaria¹⁵, but Luzatto has convincingly shown that, in infected heterozygous females, enzyme-normal red cells are much more likely to be parasitised by *P. falciparum* than are enzyme deficient cells⁶. These last results indicate either that merozoites preferentially infect enzyme-normal red cells or that, once infected, the enzyme-normal erythrocytes are much more likely to sustain the parasite for the 48 h requisite for maturation. Our results strongly favour the latter interpretation. Although previous studies of the effect of vitamin E deficiency on the expression of malaria have yielded conflicting results¹⁵⁻¹⁷, we find a markedly slower rate of development of infection in vitamin E-deficient animals. In these mice, the preferential occurrence of parasites within young, oxidant-resistant erythrocytes suggests that this protection is mediated by the premature haemolysis of oxidant sensitive red cells.

In areas where malaria and malnutrition coexist, it may be advisable to administer antimalarial therapy along with dietary supplements.

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Selective contact-dependent cell communication

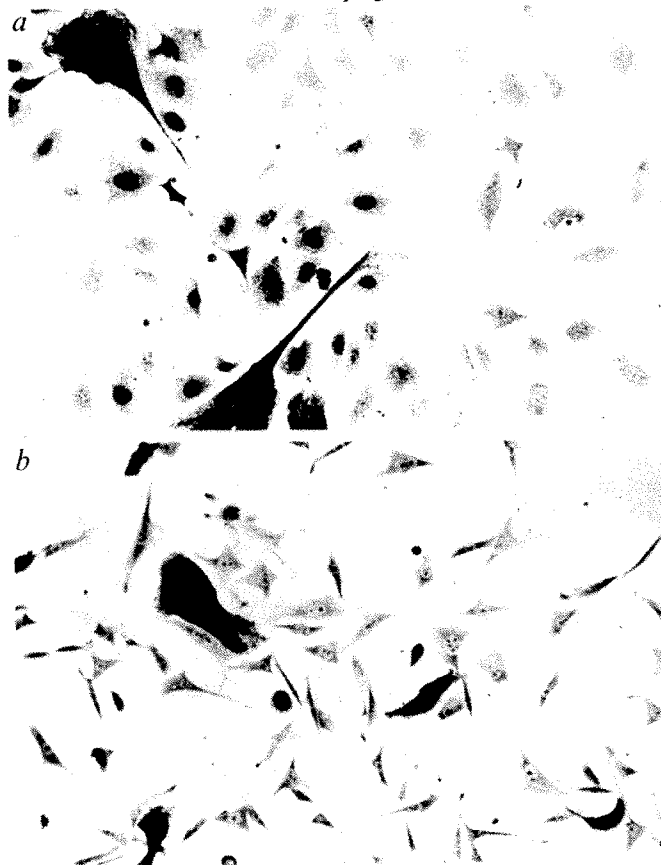
INTERACTIONS between animal cells in multicellular organisms are probably important in the coordinated control of growth and function during the normal and abnormal development of specific tissues and organs. Although much of the coordination is lost when cells are separated from each other and grown in tissue culture,

direct communication can be detected between cultured cells which may be electrically coupled^{1,2}, and which can show metabolic co-operation^{3,4}. Indirect evidence suggests that this direct exchange of ions or small molecules between touching cells, occurs through gap junctions⁵⁻⁷. We have been interested in the question of whether fibroblasts and epithelial cells from the same tissue can interact directly and whether such an interaction is important in the growth of epithelial tumours such as carcinomas of the breast. Although the two cell types are separated by a basement membrane in the normal adult breast, the invasion of the basement membrane, characteristic of malignant cancer cells, allows contact to occur between epithelial cells and fibroblasts. In this situation, the direct communication between the cell compartments of the two types of cell could have a profound effect on the growth and development of the tumour. We show here that normal breast fibroblast and epithelial cells do not directly interact with each other even when they are not physically separated, and speculate that loss of this specificity of communication may be a factor in the development of neoplasia.

Since it has proved difficult to grow the tumour cells from primary human breast carcinomas^{8,9}, we have begun by examining contact-dependent communication between human mammary fibroblasts (HumF) cultivated from explants of terminal ducts, and human mammary epithelial cells (HumE) cultured by the spillage technique from benign tumours^{8,9} or from human milks^{10,11}. Primary culture of both these cell types were thus obtained without enzyme digestion of the tissue. A cell line, MCF-7, derived from a pleural effusion from a patient with breast cancer^{12,13} was also included in the study.

Metabolic cooperation, as it was originally described by Subak-Sharpe, Burke and Pitts^{3,4}, refers to the correction

Fig. 1 a, Transfer of radioactivity from donor lens cell to recipient BHK. b, No transfer of radioactivity from donor HumE cell to recipient BHK. Method as described in Fig. 2 with the modification that cells were exposed to film for 2-4 weeks before developing.



	Donors					
	Lens	RL	BHK	HumF	HumE	MCF-7
Recipients	Lens	+	+	+	+	○
	RL	+	+	○	+	○
	BHK	+	+	+	○	○
	HumF	+	○	+	○	○
	HumE				+	
	MCF-7	○	○	○	○	○

Fig. 2 Primary cultures of HumE and HumF were grown in medium 199 supplemented with hydrocortisone ($5 \mu\text{g ml}^{-1}$), insulin ($10 \mu\text{g ml}^{-1}$) foetal calf serum (15%) and human serum (20%). MCF-7 cells were grown in 199 medium supplemented with calf serum (10%) and insulin ($10 \mu\text{g ml}^{-1}$). Calf lens were grown in 199 with foetal calf serum (10%) and all other cell lines were grown in Dulbecco's modified Eagles medium (DEM) containing 10% foetal calf serum. All cells were grown in Nunclon plastic dishes; those for growth of HumE were coated with collagen which does not affect direct cell interactions between communicating cells (J.T.-P., unpublished). Cells were suspended by exposure to trypsin (0.05%) in isotonic Tris buffer (0.02 M) containing EDTA (0.2%). Donor cells were plated in 2 ml of medium at 3×10^3 cells per dish in the medium indicated above, and after overnight growth, medium was removed and $4 \mu\text{Ci}$ of $5\text{-}^3\text{H}$ -uridine (48 Ci mmol^{-1}) were added for 3 h in 2 ml of DEM containing 10% foetal calf serum. The donors were then washed with DEM ($\times 3$) and recipients (2.5×10^5 per dish) were added in fresh DEM+10% foetal calf serum and co-cultured for 3 h in medium without label. The cells were fixed and processed for autoradiography and developed after 7–14 d. Number of grains in donors were between 300 and 2,000. Grains were counted in 150 recipients directly in contact with donors (touching) and in 150 recipients in contact with neither donors nor other recipients (non-touching). HumE cells have been used as donors for all combinations except HumE–HumE because of the limitation in number of cells available. This was considered to be reasonable since all cell combinations tested in a reciprocal manner gave the same result either way.

of a mutant phenotype by co-culture with wild-type cells. Using this method we could follow cell-cell interaction between HumE cells and a mutant fibroblastic cell line TG1 (a strain of polyoma transformed BHK cells lacking hypoxanthine guanine phosphoribosyl transferase). Though TG1 cells are known to communicate with a large variety of fibroblastic cells from several species, our experiments showed that there appeared to be no direct interaction between HumE and touching TG1 cells, suggesting that communicating junctions were not formed between the two cell types. This method is limited, however, to measuring cell interactions which involve mutant fibroblast lines and tests of reciprocity cannot be carried out. Here, we report the application of a method, recently developed by Pitts and Simms¹⁴ for the measurement of direct communication between freshly cultured cells of the same type, and of different types, without the use of mutants.

Donor cells are prelabelled with $5\text{-}^3\text{H}$ -uridine and the transfer of label to the acid-insoluble fraction of unlabelled recipients during subsequent co-culture is followed. Because the $5\text{-}^3\text{H}$ -uridine in the pools of the donor cells is rapidly converted to high molecular weight material¹⁴, the formation of junctions and transfer of radioactive label has to occur within the first few hours of removal of the label in order to be detected. In the experiments reported here, labelled donors have been co-cultured for 3 h with unlabelled recipients as recommended by Pitts and Simms. In addition to the cells from the breast, we have examined a rat liver line (RL) and primary cultures of calf lens (prepared from the explanted lens capsule) as other examples

of epithelial cells and BHK 21 cells as an example of a fibroblastic cell line.

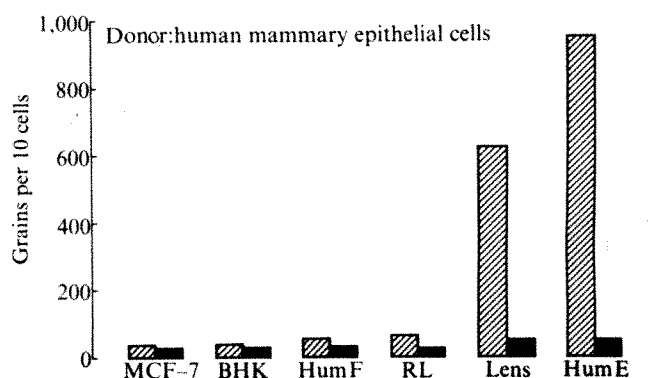
Qualitatively, examples of positive and negative communication can be seen in Fig. 1. To quantitate the transfer of radioactivity, cells were exposed to stripping film for shorter periods of time than that used for the autoradiographs in Fig. 1 so that grains could be counted. For each cell combination, three separate experiments were done using five duplicate plates in each experiment and grains were counted in 10 recipients touching donors, and in 10 recipients touching neither donors nor recipients. Thus the average number of grains in touching and non-touching recipients is calculated in each case from 150 values. The results are presented in Figs 3 and 4 and summarised in Fig. 2.

From Figs 2 and 3 it can be seen that while HumE cells communicate directly with other HumE cells (or lens cells) and HumF with HumF (or lens) no such direct communication could be detected between HumE and HumF. Previous work following direct interactions between cell lines has indicated that cells which can form communicating junctions do so without specificity as to cell type¹⁵. The results reported here demonstrate, under the conditions of the experiment, a specificity in the ability of cells to communicate directly with other cell types, and we can refer to HumE and HumF as "selective communicators". The RL cells are also "selective communicators" (Fig. 4) in that, while communicating through contact with other RL (or lens cells), they do not under the same conditions donate ^3H -uridine to HumF. However, when a fibroblastic cell line, BHK, is used as recipient instead of primary cultures of HumF, some specificity is lost and a certain amount of radioactivity is transferred from the RL donor to the touching BHK recipients. The HumE cells, however, do not donate radioactive label during 3 h of co-cultivation even to fibroblastic cell lines.

A few but not all RL cells co-cultured with and touching HumE donors showed an increased grain count over non-touching RL. In Fig. 4 this result shows up as a small increase in the average number of grain counts in touching over non-touching cells, and is represented as positive and negative in Fig. 2. The result suggests that communicating junctions can be formed between the two types of epithelial cells but not with the ease and rapidity with which they are formed between epithelial cells of the same type.

Calf lens cells are unique among the cells we have examined in that they donate radioactivity when prelabelled with $5\text{-}^3\text{H}$ -uridine to all the cells tested, (except MCF-7—see below) that is, they are "non-selective communicators". Thus there is not a general failure of all cells of epithelial origin to communicate directly with fibroblasts. The readiness

Fig. 3 Selective communication of HumE cells with other cell types. For method see legend to Fig. 2. Hatched bars, grains in touching recipients; black bars, grains in non-touching recipients.



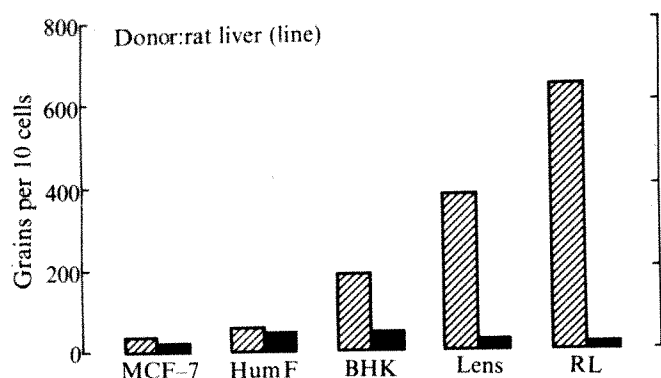


Fig. 4 Selective communication of RL cells with other cell types. Hatched bars, grains in touching recipients; black bars, grains in non-touching recipients.

with which the lens cells form communicating junctions may well be related to the abundance of gap junction proteins which are found in their membranes¹⁶.

Metabolic cooperation studies as well as electrical resistance measurements have shown that L cells and some tumour cells and cell lines do not form communicating junctions either with themselves or any other cells^{17,18}. MCF-7 cells resemble L cells in that they seem to be unable to communicate directly either with themselves or with any other cells tested.

The data reported here demonstrate that the intracellular pools of uridine or its nucleotide¹⁴ in HumE cells do not readily communicate directly with the corresponding intracellular pools in HumF cells under conditions where there is exchange between the pools of cells of the same type. This suggests an important possibility, namely, that in certain mixed cultures such as HumE and HumF, two cellular compartments may exist which do not interact by transfer of molecules. Indeed in mixed cultures of these two cell types, the cells grow physically separated from each other. If the results can be translated to the *in vivo* situation, they suggest that even if the basement membrane did not physically separate the two types of cell, they might form junctions only with cells of the same type. Such a specificity governing interactions between different cell types could be an important factor in the normal regulation of growth in multicellular organisms and further studies on interactions between cells in other tissues and organs are indicated.

Our results have been obtained with cells obtained from normal or hyperplastic breast and as yet we do not know whether the primary carcinoma cells behave like the normal epithelium. It has been suggested by Loewenstein¹⁹ that the complete loss of the ability to directly communicate with other cells, which is observed in some tumour cells (including the MCF-7 line used here) could account for the lack of response in these cells to normal growth control signals which may come through direct cell contact. The existence of specificity in direct cell-cell communication, as demonstrated here, opens up the possibility, which can be experimentally tested, that a loss of specificity in cell-cell interactions could be associated with abnormal cell growth.

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Specificity of junctional communication between animal cells

MANY types of animal cells, in culture and *in vivo*, form intercellular junctions which are freely permeable to small cellular ions and molecules but not to macromolecules¹⁻¹⁰. These junctions are probably the gap junctions seen by electron microscopy¹¹. Cells coupled by such junctions share their metabolites and small control molecules and respond jointly to changing demands on metabolism. Intercellular control of enzymic activity^{12,13} and metabolic interdependence causing controlled cell proliferation⁷ have both been observed in mixed cell populations in tissue culture. These integrative effects of junctional communication make a tissue behave as a homogenous unit rather than a collection of different cells.

The ubiquitous occurrence of gap junctions *in vivo* suggests that junctional integration is a widespread phenomenon, but one can conceive of instances where integration is a disadvantage and independence is important. Such situations would require specificity of junctional communication to functionally isolate different cell types either within the same tissue or in different contiguous tissues.

Examination of cultured vertebrate cells for their ability to form intercellular junctions has, until now, revealed only two classes. Cell types in one class (which includes about 90% of the cell types so far examined) form junctions with all other members of the class and with no detectable tissue or species specificity (ref. 9 and B. Kukulska, P. Ferry and J. D. P., unpublished). Cell types in the other class appear to be genetically incapable of junction formation and so never form junctions either among themselves or with any other cell type^{7,14,15}. However, in this paper we describe a tissue culture system which is different from these others in that it shows a form of specificity of junction formation.

Rat liver cells (BRL cells¹⁶) formed junctions rapidly and extensively with each other. Hamster fibroblasts (BHK21/13 or C13 cells¹⁷) similarly formed junctions very efficiently among themselves⁷. But in mixed cultures junctions were not formed or formed only very slowly, between BRL and BHK cells.

Uridine nucleotide exchange can be used as an assay of intercellular junction formation. Pitts and Simms^{9,10} have shown that uridine nucleotides, but not RNA, are freely transferred between cells joined by gap junctions. However, because nucleotides cannot cross the normal cytoplasmic membrane, they are not transferred to cells which are not in contact or to cells which are unable to form gap junctions.

Donor cells were prelabelled for 3 h with 5-³H-uridine and washed with unlabelled medium. Such donor cells contain 5-³H-uridine nucleotides and ³H-RNA but no detectable 5-³H-uridine. Unlabelled recipient cells were added to the washed donor cells and the mixtures co-cultured. After

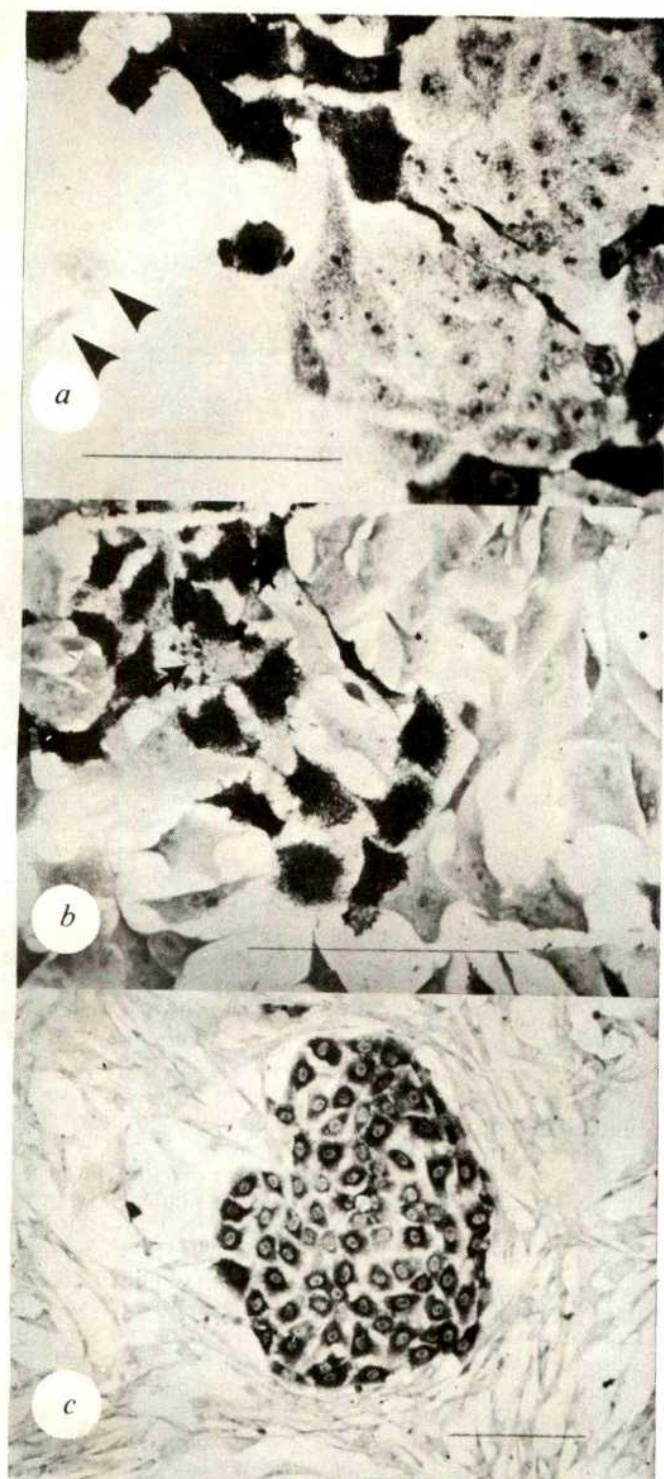


Fig. 1 Donor cells (5×10^4) in 5 ml Eagles medium (Glasgow modification, supplemented with 10% calf serum) were grown overnight at 37°C in 5 cm plastic dishes containing 13 mm diameter sterile coverslips. The cells were labelled for 3 h with $5\text{-}^3\text{H}$ -uridine ($5 \mu\text{Ci ml}^{-1}$; 27 Ci mmol^{-1}), then washed four times with unlabelled medium. Unlabelled recipient cells (5×10^5) were added to the prelabelled washed donor cells and the mixtures co-cultured for 3 h (a,b) or 18 h (c). The cultures were fixed with formal-saline. The coverslips were washed for 5 min at 0°C twice in 5% TCA and twice in water and then rinsed in ethanol. The dried coverslips were mounted on microscope slides and processed for autoradiography (Ilford L4 emulsion). The autoradiographs were stained with Giemsa. Heavily labelled cells are donor cells and unlabelled or lightly labelled cells are the added recipients. a, BRL donors to BRL recipients (note two unlabelled recipient cells not in contact with donor cells, arrowed); b, BRL to C13 (note one labelled recipient, arrowed); c, BRL to C13 (showing island of epithelial BRL cells). Scale bars: $50 \mu\text{m}$.

co-culture, the cells were fixed, acid washed and processed for autoradiography. Light-labelling over recipient cells due to uridine nucleotide transfer from the heavily labelled donor cells indicates junction formation. For details of method see description of Fig. 1 and ref. 10.

Junctions were formed between all BRL cells in contact (Fig. 1a) and between all C13 cells in contact. However, under the same conditions, junction formation between BRL and C13 cells was very rare even though they appeared by light microscopy to be close enough to make contact.

The extent of nucleotide transfer in the co-cultures was quantitated by counting grains over recipient cells. Primary recipients (recipient cells in direct contact with labelled donor cells) were then classified as labelled or unlabelled. Secondary recipients (recipient cells in direct contact with labelled primary recipients) were similarly classified.

The percentages of primary recipient cells labelled (the extent of transfer) in both homologous and both heterologous donor-recipient mixtures are given in Table 1. No instance was found of an unlabelled recipient BRL cell in contact with donor BRL and only two of 328 recipient C13 cells in contact with donor C13 cells were unlabelled. This very extensive junction formation is similar to that found with a wide variety of cell types^{6,7,10}.

A new situation arose, however, when junctional communication was examined in the heterologous mixtures. Even though there was close apposition between donor cells and potential recipients, detectable transfer occurred between less than 6% of the cell pairs examined after 3 h co-culture (Table 1). In the few cases where there was transfer (5.4% for BRL to C13 and 4.9% for C13 to BRL) the amount of transfer was less than that between cells of the same type, but not greatly so (Table 2 and compare the number of grains over the one labelled recipient in Fig. 1b with those over the labelled recipients in Fig. 1a). In the heterologous cultures, homologous transfer always occurred from every labelled primary recipient cell (Table 1).

The extent of interaction in the heterologous mixtures appears to be a function of the time of co-culture. If the donor and recipient cells were co-cultured for only 1 h the extent of interaction was less than 2% (1.9% for BRL to C13, 1.2% for C13 to BRL), but after 18 h it increased to almost 50% (44% for BRL to C13, 41% for C13 to BRL). In the homologous mixtures the extent of interaction was 100% at both 1 h and 18 h.

It seems that BRL cells do not form junctions readily with C13 cells but, in at least some cases, they do after

Table 1 Specificity of junction formation

Donor cells	% Recipient cells labelled			
	Primary recipients	Secondary recipients	Primary recipients	Secondary recipients
BRL	100	100	5.4	100
C13	4.9	100	99.4	100

Donor cells (2×10^4) were labelled for 3 h with $5\text{-}^3\text{H}$ -uridine ($0.2 \mu\text{Ci ml}^{-1}$) and then washed four times with unlabelled medium (see Fig. 1). Unlabelled recipient cells (8×10^5) were added and the mixtures co-cultured for 3 h. After fixing, washing and processing for autoradiography, grains were counted over labelled recipient cells in direct contact with donor cells. The heavily labelled donor cells are readily distinguished from the lightly labelled recipients. Primary recipients are recipients in direct contact with a donor cell. Secondary recipients are recipients in direct contact with a primary recipient cell. Recipient cells with < 5 grains per cell were classified as unlabelled, with > 30 grains per cell as labelled. The background count was < 4 grains per cell. Cells with grain counts between 6 and 29 were ignored (7 out of 1,264 counted). More than 200 donor-recipient pairs were examined in each mixture (except for the secondary recipients in the heterologous mixtures where insufficient examples could be found and only 70 primary recipient-secondary recipient cell pairs were counted).

prolonged contact. Once junctions have been established, the rate of nucleotide transfer is similar to that between cells of the same type. The grain counts over primary recipients are lower when the cell types are different (Table 2) but if the time course of junction formation in these heterologous co-cultures is taken into account, then the average grain counts (average for cells which formed junctions early and cells which formed junctions later in the 3 h period) suggest similar rates of transfer.

Table 2 Extent of nucleotide transfer

Grain counts over primary recipient cells	
BRL to BRL	63 ± 19
BRL to C13	48 ± 20
C13 to C13	88 ± 29
C13 to BRL	53 ± 16

For experimental conditions see Table 1. The figures are mean values of grain counts over either 200 (BRL to BRL and C13 to C13) or 70 (BRL to C13 and C13 to BRL) primary recipient cells with standard deviations.

Junctional communication between BRL and C13 cells, once established, appears to be similar to that in other systems and it is possible that the junctions are identical. It may be, therefore, that the frequency of cell-cell contact and not a difference in junctional subunits underlies the specificity of junction formation. The frequency with which the membranes of homologous cell pairs come close enough together to allow junction formation may be much higher than that possible for BRL-C13 pairs.

In cell systems where junctions form quickly and extensively, communication is maintained (even through cell division) until the cells are physically separated (for example by trypsinisation or mechanical means). However, in the heterologous cell systems described here, lightly labelled cells (recipients) not in contact with a more heavily labelled cell can be found occasionally (0.8% of labelled recipients after 18 h co-culture). This ability to break communication, even at this very low rate, is strikingly different from the homologous systems where all labelled recipient cells were in contact with heavily labelled cells. This may reflect the relative weakness of the heterologous cell-cell contact.

In both mixed and unmixed cultures the epithelial-like BRL cells tend to form islands or domains and thus maintain maximum cell-cell contact. In the mixed cultures these islands are not invaded by the fibroblasts which suggests that the BHK cells cannot satisfy the BRL cells' requirement for intercellular contact. This inability to form adequate contact between the two cell types may explain both the culture morphology and the specificity of junction formation.

This paper describes a type of specificity in the formation of junctions between cells in tissue culture. The accompanying paper¹⁸, using cells in primary culture, with similar results, suggests the specificity we have analysed may be a general phenomenon.

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Response to epidermal growth factors of cultured human mammary epithelial cells from benign tumours

In a pilot study on the culture of unselected human breast tumours it was found that fibroadenomas which were disrupted mechanically (without enzyme treatment) consistently gave rise to epithelial cell cultures uncontaminated by fibroblasts¹. These epithelial cells, which were termed E cells, could not be distinguished from the major class of epithelial cells isolated from lacteal secretions of healthy women^{2,3}. It is not known whether the fibroadenoma yields epithelium which is truly normal (and is thus a fibroma), or whether the epithelium is abnormal in a way not yet identified. Nevertheless, the reproducibility of the cultures from these tumours, together with ease of storage at low temperature, makes them suitable for detailed studies on mammary epithelial growth, and for comparison, not only with lacteal secretion cells, but with the more heterogeneous cultures from carcinomas. We describe here some of the growth characteristics and requirements of epithelial cells from fibroadenomas, and in particular their response to epidermal growth factor⁴, but lack of response to more familiar mammotrophic hormones.

Cultures were made from stored frozen aliquots of uncultured cell suspensions from mechanically disrupted fibroadenomas removed in the ICRF Breast Unit at Guy's Hospital. The suspensions, which consisted predominantly of clusters of 10-100 cells were distributed in growth medium (see legend to Fig. 1) to give 100-500 clusters per 30 mm dish as required (see legend Fig. 1). Experiments were repeated with fresh cultures from the same or different tumours. Except for cell yield, there was little variation among fibroadenomas, nor among replicate or successive cultures from the same tumour. Viability was unaffected by storage in liquid nitrogen for up to 8 months.

When incubated at 37 °C, 5-15% of the cell clusters attached and spread to give characteristic smooth-edged islands of tightly adjoining cells typical of the E cells known to exhibit epithelial junction systems and microvilli. Islands of fibroblast like cells were found in cultures from only two out of 50 tumours, and no mixed (epithelial-fibroblastic) islands were seen. Though the epithelial islands were uniform in appearance however, we have no evidence that they are all identical or individually homogenous.

The islands enlarged during the following 3 weeks with approximately a 2-d average doubling time to reach a maximum of about 10⁵ cells. After 3 weeks in culture, however, growth consistently came to a halt, as shown by lack of further enlargement of islands, and absence of DNA synthesising cells detected by autoradiography after 48 h exposure to ³H-thymidine. A similarly limited growth period has been found for E cells from lacteal secretions³. Subculture of the growing cells by suspension in 0.025% trypsin gave similar islands (plating efficiency 2% and 7% in two experiments) but did not extend the total growth period.

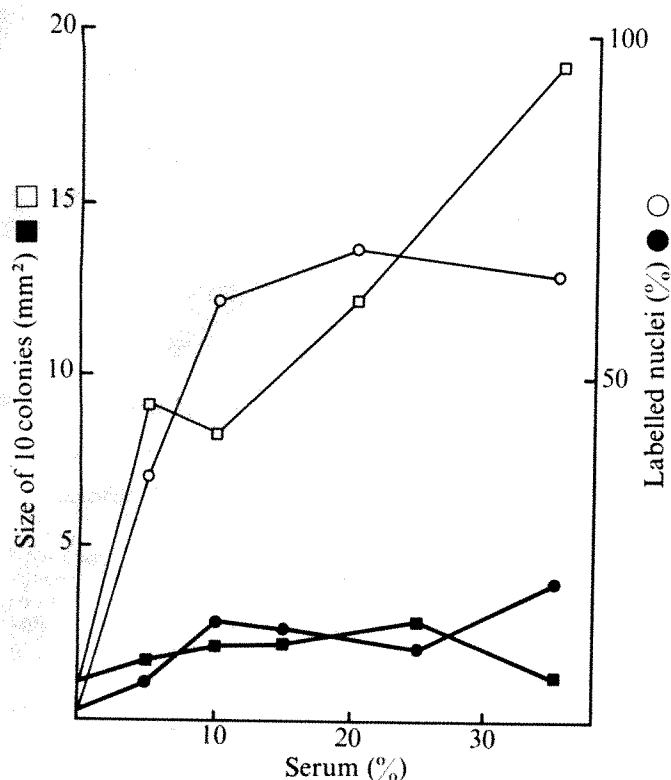


Fig. 1 Response of mammary epithelial cells to human serum and foetal calf serum. Fibroadenomas were disrupted mechanically and the resulting suspension filtered through gauze and allowed to sediment on the bench for 30 min. The loose deposit was suspended in medium with 10% dimethyl sulphoxide to give 10^3 to 10^4 cell clusters per ml and stored in 1 ml aliquots at -160°C . For culture, samples were thawed and distributed in 30 mm dishes to give several hundred clusters per dish in 2 ml volumes of the following medium: medium 199 with Earle's salts, buffered with sodium bicarbonate (3.7 mg ml^{-1}) and containing L-glutamine ($100\text{ }\mu\text{g ml}^{-1}$), glucose (1 mg ml^{-1}), streptomycin ($100\text{ }\mu\text{g ml}^{-1}$), penicillin (100 U ml^{-1}), supplemented with hydrocortisone ($5\text{ }\mu\text{g ml}^{-1}$), insulin ($10\text{ }\mu\text{g ml}^{-1}$) unactivated filtered ($0.22\text{ }\mu\text{m}$ millipore) foetal calf serum (15%) and pooled human serum (20%). Medium was changed twice weekly. After 14 d single cultures were washed twice in serum-free medium, changed to 1 ml of medium 199 (with hydrocortisone and insulin as above) and to either human serum (open symbols) or foetal calf serum (closed symbols) in the concentrations shown. After a further 2 d ^3H -thymidine ($2\text{ }\mu\text{Ci ml}^{-1}$, 20 Ci mmol^{-1}) was added for 48 h. The labelling index (%) was determined by autoradiography and based on counts of 800 to 2,000 cells distributed in 10 randomly distributed islands per plate. Colony size was estimated from the total area of the same 10 colonies. Cell density was not affected by island size.

The studies reported here were carried out between days 9 and 18 of primary culture.

In our earlier study the standard growth medium contained 15% foetal calf serum, but an additional 20% human serum was added when it was observed that epithelial growth was enhanced. To investigate further the serum requirements, growing cultures of E cells were washed in serum-free medium and replaced in medium without serum, or with varying quantities of either human or foetal calf serum. As judged by labelling index, or cell numbers derived from island size (Fig. 1) E cell growth is maintained in human serum, proportionately to dose, but there was little response to calf serum. Various batches of both sera were tested with the same results. Mammary fibroblasts, on the other hand, obtained from enzyme-digested fibroadenomas, or in rare instances from mechanically disrupted tumours, responded equally well to foetal calf serum and human serum (data not shown). Since the response of epithelium to human serum was not inhibited in mixtures with calf serum, we assume that the latter is defective in essential growth factors for epithelium. The requirement is

not species specific however because adult swine serum (but not adult cow serum) will also support human mammary cell growth.

The activity of human serum was considerably reduced by absorption for 1 h with activated charcoal (Norite 5 \times 2, 10 mg ml^{-1} ; Harrington, London). In a preliminary attempt to identify the active constituents, certain hormones were added to washed cultures of E cells in place of serum. Oestradiol (1 and 10 ng ml^{-1}), human and ovine prolactin (0.1 and $1\text{ }\mu\text{g ml}^{-1}$), insulin ($10\text{ }\mu\text{g ml}^{-1}$), and hydrocortisone ($5\text{ }\mu\text{g ml}^{-1}$) were unable to maintain DNA synthesis when used alone, or in various combinations, in cultures from several different fibroadenomas. Mouse epidermal growth factor (EGF), however, consistently maintained DNA synthesis in cultures from all fibroadenomas tested. Figure 2 shows that 1 ng ml^{-1} ($1.7\times 10^{-10}\text{ M}$) was effective and the response increased up to 10 ng ml^{-1} . At higher concentrations the response was not increased proportionately (and sometimes fell) so 10 ng ml^{-1} was routinely used. When 0.1% serum was added (insufficient to maintain DNA synthesis alone) there was a small enhancement at the lower EGF concentrations.

Table 1 gives data from three experiments and shows that EGF increases the proportion of DNA synthesising cells, as well as overall synthesis measured by ^{14}C -thymidine incorporation. It will be seen that the response to EGF is unaffected by hydrocortisone, with or without insulin, but is slightly enhanced by 0.1% serum as already shown. Other experiments have shown that insulin alone, oestradiol, and prolactin (concentrations as above) or bovine serum albumin ($2.5\text{ }\mu\text{g ml}^{-1}$) have no effect on response to EGF. EGF is known to stimulate mouse and human fibroblasts, so the substantial response of mammary fibroblasts, also shown in Table 1, is not surprising.

Thus far we have only shown that human serum and

Fig. 2 Response of human mammary epithelial cells to epidermal growth factor (EGF). Fibroadenoma cultures as in Fig. 1. After 12 d in growth medium cultures were washed twice in serum-free medium and EGF was added in doses shown to pairs of dishes in 1 ml volumes of medium 199 (with hydrocortisone and insulin) either with (squares) or without (circles) 0.1% human serum. After 3 d medium was changed, with same additions, and ^{14}C -thymidine ($0.05\text{ }\mu\text{Ci ml}^{-1}$, 50 mCi mmol^{-1}) was added for a further 2 d before acid extraction and scintillation counting, shown separately for each culture.

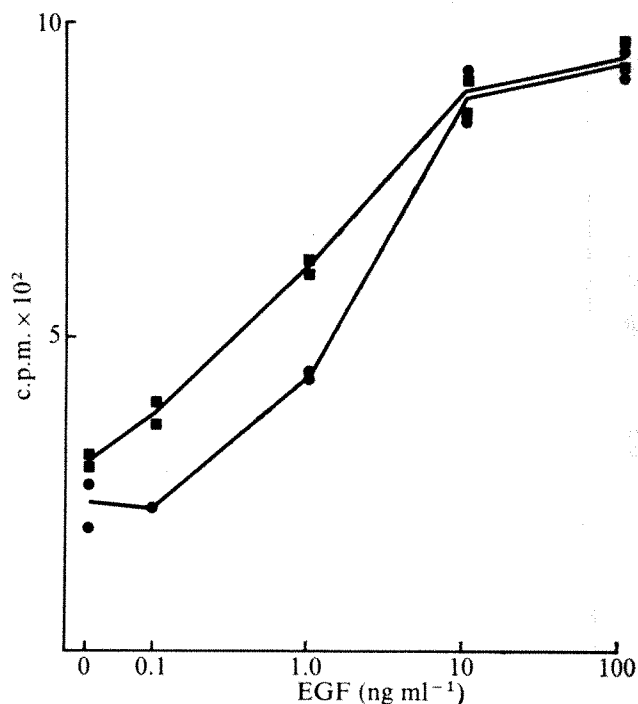


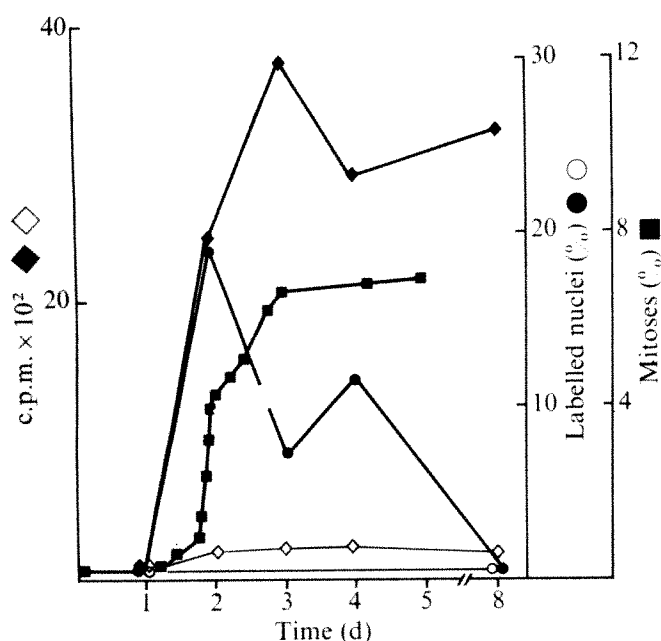
Table 1 Effect of epidermal growth factor, hydrocortisone, and insulin on thymidine incorporation by human mammary epithelial cells and fibroblasts

Additions		Experiment 1 (% labelled) Epithelium FA T99 Growing	Experiment 2 (c.p.m.) Epithelium FA T286 Growing	(a)	Experiment 3 (c.p.m.) Epithelium FA T232 Growing	Quiescent	Experiment 3 (c.p.m.) Fibroblast MP N2F Growing	Quiescent
nil		0.8	17; 22	40; 38	—	—	—	—
Hydrocortisone	Insulin	0.1	26; 44	124; 28	47; 50	75; 59	169; 129	225; 130
—	EGF	17.7	453; 486	778; 597	—	—	—	—
Hydrocortisone	Insulin	14.0	388; 411	629; 388	321; 297	208; 235	2,544; 2,400	3,205; 2,389
Hydrocortisone	—	—	13; 48	26; 33	—	—	—	—
Hydrocortisone	EGF	—	440; 452	832; 597	—	—	—	—
Human serum	—	35.6	4,303; 4,483	—	4,680; 5,704	690; 754	29,920; 30,499	16 795; 16,903

Epithelial cultures as in Fig. 1 from three separate fibroadenomas; fibroblast cultures by plating dispersed cells after collagenase and hyaluronidase digestion of normal mammary tissue removed at mastectomy (10⁵ cells per 30 mm dish). After 9 d in growth medium the cultures were washed twice with serum-free medium and treated as follows: for growing cultures 1 ml medium 199 was added, with the additions shown, for 3 d. The medium with the same additions was then replaced for a further 2 d with ³H-thymidine or ¹⁴C-thymidine. In experiment 2a 0.1% human serum was present throughout. For quiescent cultures medium 199 (with hydrocortisone and insulin) was added for 3 d to all cultures, then replaced for 2 d with the additions shown and ¹⁴C-thymidine. The labelling index (experiment 1) was estimated by autoradiography and counts on all labelled nuclei in 10 randomly selected colonies, comprising at least 3,800 cells. Incorporation of ¹⁴C-thymidine (experiments 2 and 3) in duplicate cultures, shown as counts per minute per culture above background (10 min counts, background exp. 2: 16 c.p.m., exp. 3: 29 c.p.m.). Cell numbers were not high enough for estimation of DNA. Concentrations of additions were as follows: hydrocortisone 5 µg ml⁻¹, insulin 10 µg ml⁻¹, mouse epidermal growth factor 10 ng ml⁻¹ (25 ng ml⁻¹ in exp. 1), unactivated pooled adult human serum 20% (35% in exp. 1).

EGF can maintain DNA synthesis in already growing cultures of mammary epithelial cells. In medium 199 alone, with or without insulin and hydrocortisone, such cells stop synthesising DNA and dividing, and remain quiescent with a labelling index (48 h exposure to ³H-thymidine) of less

Fig. 3 Time course after restimulation of quiescent mammary epithelial cells with epidermal growth factor (EGF). Fibroadenoma cultures as in Fig. 1. After 10 d in growth medium, cultures were washed twice in serum-free medium, and left in 1 ml of medium 199 (with hydrocortisone and insulin) for 3 d. The medium was then changed and EGF (10 ng ml⁻¹) added to about half the cultures. The remainder were controls without EGF. At this stage, one set of cultures, with and without EGF, also received ¹⁴C-thymidine, and thereafter one culture was removed each day for acid extraction and scintillation counting, shown as cumulative incorporation per culture. The other set, with and without EGF, was exposed to ³H-thymidine added on successive days for 24 h periods, before autoradiography and estimation of labelling index (based on counts of at least 3,000 cells distributed in six randomly selected islands). Closed symbols: with EGF; open symbols: without EGF. Mitoses were determined by time lapse cinematography on one culture with EGF and are shown as the cumulative number in a single confluent area within one island and containing about 400 cells.



than 1% for several weeks without morphological evidence of cell damage. Table 1, experiment 3, shows that both serum and EGF can reinitiate DNA synthesis in such quiescent cultures of E cells (and also mammary fibroblasts), while in Fig. 3, a time course from another experiment, shows that reinitiation of DNA synthesis occurs 24 to 48 h after addition of EGF and is accompanied by mitosis, but is limited to one cycle. While human serum or EGF invariably maintain DNA synthesis in growing culture, however, the response of quiescent cultures to restimulation was variable and in the case of EGF sometimes undetectable. Lack of reinitiation was not a feature of particular tumours, or time in culture, and is at present unexplained.

EGF which is a stable and highly purified polypeptide hormone isolated by Cohen and his colleagues^{7,8} is mitogenic for murine⁶ and human fibroblasts^{7,8} for mouse mammary epithelial cells in organ culture⁹ and rat mammary epithelium in cell culture (P. S. Rudland, R. D. Hallows, H. Durbin, and D. Lewis, unpublished). A polypeptide with similar biological properties has recently been isolated from human urine¹⁰. In these systems serum or albumin is also required for EGF activity, whereas in the cultures described here EGF invariably maintained, and, less regularly, reinitiated, DNA synthesis in defined medium in the absence of added protein. Admittedly, serum might have been carried over despite repeated washing of the cultures, but is unlikely to have been an important factor since re-addition of serum had little effect on the EGF response. Though EGF could not replace the maximum effect of human serum (see Table 1), thus implying the need for other factors, it was effective at a physiological level, such as reported in mouse plasma¹¹.

The lack of response of our cultures to known mammatropic hormones, such as prolactin and oestradiol, may be due to selection of an unresponsive cell, or inadequate culture conditions, perhaps even the lack of a required interacting heterologous cell. It is clear, however, that other less familiar hormones such as epidermal growth factor should also be investigated for their role in the physiology and pathology of the mammary gland.

We would stress that this report concerns mammary epithelium studied within 3 weeks, or about 10 average cell doublings, of the origin *in vivo*, and in the absence of fibroblasts. In addition to the failure to respond to foetal calf serum, which may have nothing to do with EGF, the

epithelial cells differed from human fibroblasts in their much shorter life span. This characteristic of freshly cultured epithelial cells may result from inadequate culture conditions, but alternatively it may be a feature of cells which are shed after a limited proliferative phase.

Finally, since the cells will grow alone in the absence of fibroblasts, we can conclude that interaction with stromal cells is not an absolute requirement for growth of epithelium. This does not mean that interactions with non-epithelial cells are excluded, and indeed other investigations from our laboratory (ref. 3 and unpublished data) show that fibroblasts from the breast and other sources, as well as macrophages all affect the growth of mammary epithelium. But the starting point for such studies must be a source of freshly derived epithelium, free of heterologous cells, as described here.

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Cell-cycle initiation in yeast follows first-order kinetics

THE mitotic cell cycle is an orderly sequence of events, leading to cell division. In mammalian cell lines, a single event in the cycle exhibits first-order kinetics¹, apparently dividing a cell's lifespan into a probabilistic state and a deterministic phase, termed A and B respectively. This rate-limiting event occurs during the G₁ phase^{2,3}, but its relation to the resting state and to the initiation of the cell cycle is unclear. In this paper we suggest that a probabilistic event exists in the mitotic cell cycle of yeast and that it

can be identified with the initiation of a new cycle from the resting state.

The yeast *Saccharomyces cerevisiae* signals a new cell cycle by initiating a bud. The size of the bud, as it develops, is correlated with the phase of the cell in the cycle. Temperature-sensitive cell-division cycle (*cdc*) mutants were isolated, which cannot proceed beyond a particular phase of the cycle at the restrictive temperature^{4,5}. In addition to the mitotic cell cycle which is common to both haploid and diploid strains, two other developmental pathways are observed in yeast. One pathway, conjugation, is normally an alternative to the cell cycle in haploid cells, while meiosis, the other pathway, is an alternative only in diploid cells. The three pathways intersect at a common point, termed "start", at which emergence to any one of them occurs⁶. The environmental conditions determine which developmental pathway is chosen by the cell when it is at "start".

Yeast cells may be arrested before "start" as single, unbudded cells by one of three methods^{5,6}, namely by starvation into stationary phase, by incubation at the restrictive temperature of a *cdc* mutant that arrests at this point of the cell cycle and by exposure of haploid cells to the mating hormone of the opposite mating type. We have used the two latter methods to arrest two exponentially growing populations of a haploid A strain that carries the mutation *cdc25* (ref. 7), which is a "start" mutation, and followed bud emergence after release of the blocks (Fig. 1). In both populations buds emerged asynchronously following similar first-order kinetics with a defined lag period of about 45 min (see also straight-line curve semi-logarithmic plotting of data in insert of Fig. 1). Similar results were obtained with two other "start" mutations, *cdc28* and *cdc35*. For comparison, data are given of the release of the temperature block of another mutant, *cdc24*, which also arrests prior to bud emergence but at a later stage than "start"⁷ (Fig. 1). In the latter case buds appear with a much better synchrony than after

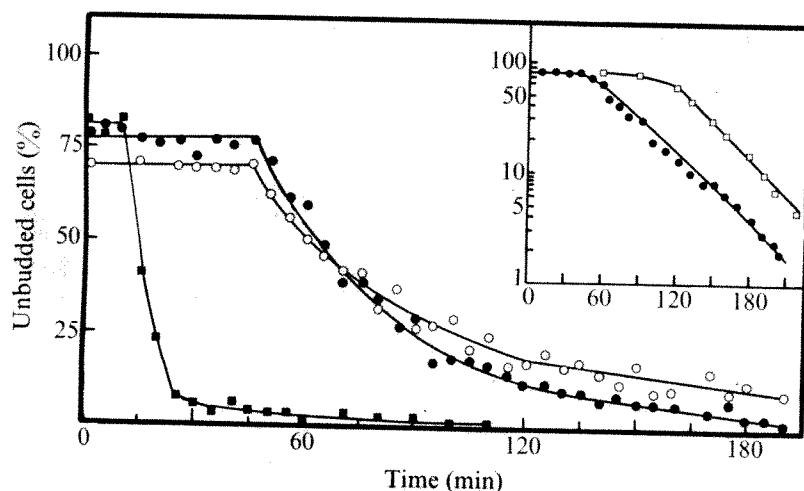


Fig. 1 Kinetics of bud initiation following release from blocks at "start" and at a later stage. The haploid strain 352/1 was derived from Hartwell's strain BR-205-2A. It is of mating type *a* and contains a temperature sensitive "start" mutation *cdc25* and the markers *ade2* and *ura1*. The haploid 337, named by Hartwell 182-6-3A is of mating type *a* and contains the mutation *cdc24* and the markers *ade1*, *ade2*, *ura1*, *lys2*, *his7*, *try1*, *gal1*. The cells were grown in YEPD medium, containing 10 g yeast extract, 20 g bacto peptone, and 20 g dextrose in 1 l of distilled water. Exponentially growing cells were blocked before "start" by a 3.5-h incubation at the restrictive temperature 36 °C in a shaking bath, or by a 2.5 h incubation at 25 °C in a medium where *a* cells were previously grown to high density and removed from the medium by centrifugation. The temperature block was released by transferring the culture to 25 °C and the *a*-factor block was removed by centrifugation and resuspension in fresh YEPD. In order to separate cell aggregates, the cultures were sonicated upon release from the blocks with the microtip of sonifer B-12 (Branson) for two 5 s periods (power 125 W). After sonication the cells were diluted to 2×10^6 cells ml⁻¹ and plated on YEPD solid medium (2% agar) at 25 °C. Samples were scored at 5 min intervals. The percentage of single unbudded cells was determined by counting at least 300 cells for each sample under the microscope. (●) strain 352/1 (*cdc25*) following release of temperature block. (○) strain 352/1 following release of *a*-factor block. (■) strain 337 (*cdc24*) following release of temperature block. The insert shows a semi-logarithmic plotting of 352/1 following release of temperature block (●) and the appearance of the second buds in *cdc24* (□). Note: similar kinetics were obtained with 352/1 after a 6-h block at 36 °C.

the former two blocks, following a well timed course of events which is initiated uniformly in all the cells that are released from the block. The synchronous budding of *cdc24* suggests that asynchrony is not a general feature of budding or of the *cdc* mutants, but is rather a consequence of the existence of a rate-limiting step in the cycle. After passing this step, the appearance of the second buds in *cdc24* is asynchronous and follows similar kinetics to the appearance of the first buds in *cdc25* (insert of Fig. 1).

When does the rate-limiting step occur? It must be located before the *cdc24* block because release of the latter allows for synchronous pursuance of the cell cycle. With respect to the "start" point, which is defined by the *cdc25* block, the rate-limiting step could occur either after, or at this very point. However, it is difficult to monitor the "start" event because it occurs up to 45 min before bud emergence. In order to distinguish between the two alternatives, the following experiment was conducted in which the actual rate of "start" emergence was monitored. *cdc25* cells were released after being blocked at the restrictive temperature. Following different periods at the permissive temperature, samples were returned to the restrictive one. The experiment was stopped when the fraction of budded cells at the permissive temperature reached a sufficiently high value (80% budding). In each sample the frequency of buds at the time of return was compared to the frequency recorded at the end of the experiment.

One of two sets of results can be expected according to the two alternatives. The first alternative is that the rate-limiting step is located after "start" and release from "start" is highly synchronous. Provided the subcultures are incubated a minimal length of time at the permissive temperature, sufficiently long to ensure full "start" release, they should all pass through the subsequent rate-limiting step independently of temperature. Since the experiment is stopped when the rate-limiting step is almost completed (80% budding at 25 °C), all the samples should have the same fraction of buds at the end of the experiment, irrespective of the length of incubation at the permissive temperature. The second possibility is that "start" itself is the rate-limiting step. Since the mutants are temperature sensitive for "start", they can emerge at "start" only at the permissive temperature. Therefore, one would expect the fraction of budded cells at the end of the experiment to depend upon the incubation period at the permissive temperature: the longer the period at 25 °C, the lower the fraction of unbudded cells.

In accordance with the second alternative, it was found that the rate of escape from the "start" block followed first-order kinetics identical to the kinetics of bud initiation at the permissive temperature, with a difference of 10 min (Fig. 2). We conclude that "start" is indeed the first-order rate-limiting step of the cell cycle. The 35-min lag period, prior to emergence from "start", may be required for activity of the *cdc25* gene product to allow the realisation of the "start" event. Budding is observed 10 min after this realisation. An essentially similar result was obtained when the initial arrest of *cdc25* cells was by α -hormone (not shown).

Two additional points can be argued in favour of the location of the rate-limiting event at "start". The existence of such a step in the cell cycle should cause accumulation of cells before this step in any growing population. Therefore, the rate-limiting event must be situated in a phase where the trapped cells may stay viable for long periods, as required also from stationary phase cells. Indeed, stationary phase cells accumulate before "start"^{5,6}. The second point is based on the nature of the cell cycle in *S. cerevisiae*. Analysis of *cdc* mutants reveals that after "start" the cell cycle diverges into two independent

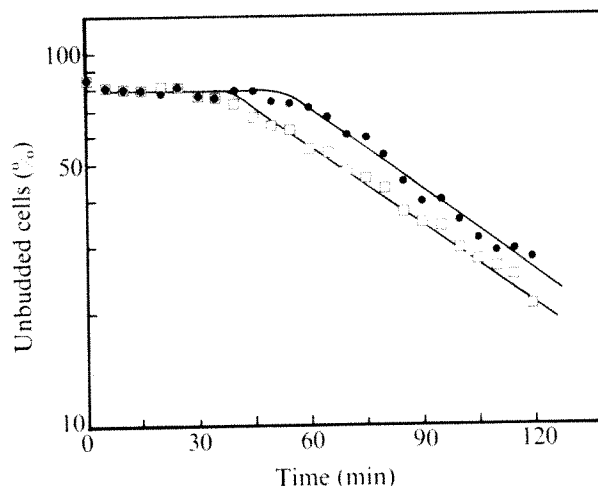


Fig. 2 A comparison between the rate of bud initiation and emergence from "start". Exponentially growing cells of strain 352/1 in liquid YEPD medium were incubated for 3 h at 36 °C. After sonication the cells were shifted to 25 °C. At 5 min intervals samples were fixed with formaldehyde (●), or shifted back to 36 °C and fixed 125 min after zero time (□). Note that the time scale applies directly to budding in the control culture (●) and to times of transfer in the shifted samples (□), where budding was scored at 125 min.

parallel pathways: One is that of DNA synthesis and nuclear division, and the other is composed of bud initiation and nuclear migration⁵. The pathways converge before cytokinesis. The rate-limiting event in the cell cycle must be situated before the divergence of the two pathways because otherwise buds would emerge without nuclear division, or, conversely, multinucleated cells would be formed.

A possible interpretation for the first-order kinetics of cell-cycle initiation is that "start" is a probabilistic event. Although each cell may have the same probability of emerging from "start", only a fraction of the culture would initiate the cycle per unit time. The "transition probability" of each cell, as termed by Smith and Martin¹, determines the magnitude of this fraction. The stage of accumulation of cells in G₁ prior to "start", can be considered the probabilistic state of the cell cycle (or "A state" of Smith and Martin), in which the cell can spend a variable length of time. The probability element in "start" release does not necessarily have to be the only factor influencing the event. It is likely that the physiological condition of the cell would also affect "start", either by imposing threshold requirements (such as a minimal cell size), or by modifying the rate constant.

It seems that "start" is the only rate-limiting step in the cell cycle of yeast. On initiating the cycle, a cell enters the deterministic phase (or "B phase" of Smith and Martin), which is an orderly sequence of events with a constant length of time. This is indicated by the behaviour of the mutant *cdc24*, which is blocked after "start" and shows synchronous bud emergence following a shift to the permissive temperature (Fig. 1). A second indication of the determinative nature of events following "start" is that bud initiation and emergence from "start" follow identical kinetics in double transfer experiments (Fig. 2). A third indication is that the formation of large buds, which occurs at a later stage of the cell cycle, follows that of small buds at an approximately similar rate (Fig. 3). That no additional rate-limiting step occurs between budding and the next "start" event is also apparent from the kinetics of emergence of second buds following *cdc24* release, which are similar to the kinetics of emergence of first buds in *cdc25* (insert of Fig. 1).

We propose that only a fraction of the cell population

is proliferating. The remainder is waiting in a resting state, equivalent to the stationary phase, and may emerge into the active cell cycle at any time with a fixed probability. Thus the doubling time of the total cell population is expected to be longer than the cycle time of the proliferating cells. The cycle time is defined as the length of the deterministic phase of the cell cycle. It can be estimated as the period between the appearances in the cell population of the first and the second buds on solid medium (Fig. 3). This value (80 ± 5 min) can be a slight underestimate of the mean cycle time because it relates to a fraction of the population that might be faster for physiological reasons (for example, newly formed cells may have a longer cycle time). Doubling time in this experiment (115 ± 10 min) was found to be 1.4 times longer than the cycle time, as expected from our proposition. Other estimates of the cycle time could be used, such as the period between the first and second peaks of small buds in liquid medium (this is an overestimate). These estimates were also found to be smaller than the doubling time.

What are the implications of the rate of cell-cycle initiation on the growth rate of the cell population? Modification of cell-cycle initiation rate by environmental conditions should change the doubling time of the population by changing the average period spent by each cell prior to "start". Indeed, when grown in PSP2 medium, where the doubling time of the population was 1.8 times longer than in YEPD medium, the rate constant of bud emergence decreased significantly (Fig. 4). Doubling time in PSP2 was 1.7 times longer than the period between the first and second buddings on solid PSP2. (PSP2 is an acetate growth medium and YEPD is a glucose medium; see details in Figs 1 and 4). In contrast to these observations with *cdc25*, the rates of bud emergence from a *cdc24*

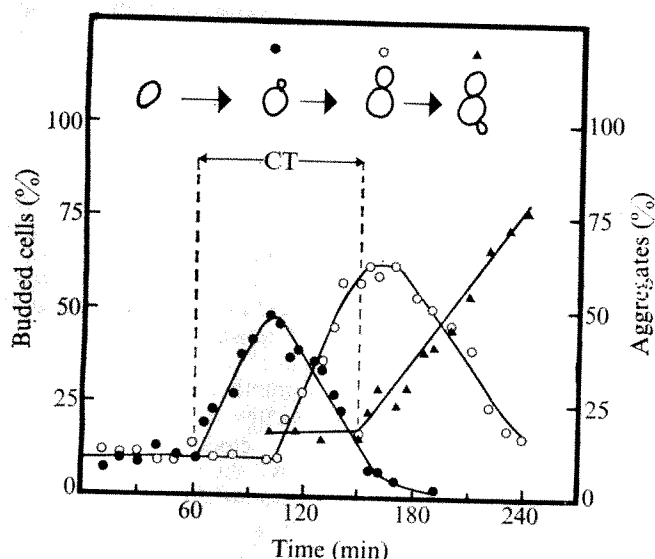


Fig. 3 Kinetics of formation of small buds, large buds and cell aggregates. The strain 452/16 is a diploid, homozygous for the mutation *cdc25*. It is also heteroallelic for *ade2* and carries the following mutations in a heterozygous constitution: *ade1*, *ura1*, *tyr1*, *his7*, *lys2*, *gal1*, *leu*, *trp*, *arg*, *met*, *iso* and *can1*. Exponentially growing cells in liquid YEPD medium were incubated for 3.5 h at 36 °C. After sonication the cells were shifted to 25 °C and plated on YEPD solid medium (2% agar). The percentages of small buds (●), large buds (○) and aggregates of three or more cells (▲) were determined. Small buds were defined as having less than half the diameter of the parent cell, large buds as having greater than half¹⁰. The cycle time (CT) was measured by the period between the appearances in the culture of the first buds and of cell aggregates of three or more cells. Doubling time at 25 °C was determined by counting the cells in a haemocytometer or by viable counts on YEPD agar plates, and was found to be 115 ± 10 min.

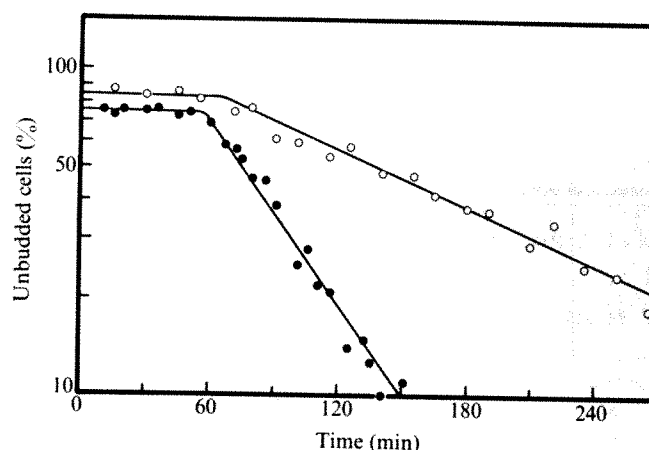


Fig. 4 Rate of bud emergence in two different media. The strain 452/16 was grown in liquid YEPD growth medium (●) or PSP2 acetate growth medium (○), containing 6.7 g yeast nitrogen base, 1 g yeast extract, and 10 g potassium acetate in 1 l of 0.05 M potassium phthalate buffer (pH 5) and supplemented with 40 mg adenine. After 3.5 h at 36 °C the cells were plated on solid media (2% agar) and shifted to 25 °C. Samples were scored as described in legend to Fig. 1. Note: after 7 h on PSP2 solid medium, 2% of the cells remained unbudded.

block in the two different media were similar (not shown). It appears that only the rate of cell-cycle initiation is modified in PSP2.

Another implication of the probabilistic nature of "start" is that it is impossible to synchronise cell populations beyond a certain level by methods employing blocks at "start". Even synchronisation attained by other methods, such as blocks at other stages or selection procedures, would be lost at the first "start" point encountered. Indeed, this has been common experience in yeast synchronisation experiments⁸ and is shown in our experiment with *cdc24* (insert of Fig. 1).

In conclusion we propose that in *S. cerevisiae* a probabilistic event occurs at the cell-cycle initiation, the "start" point, which is also the rate-limiting step of the cycle. The rate of cycle initiation can be altered by changes in the environment, thus modifying the growth rate of the population. The probabilistic event suggested for the mammalian cell cycle¹ may similarly be identified with the main regulatory point of the cycle, the "restriction point". The latter is located at the point of emergence from the resting state in *G*₁ into the proliferative cell cycle and is analogous to "start".

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EGTA and proteinase reversal of cellular aggregation of activated lymphocytes

ABUNDANT evidence indicates that cellular aggregation is essential for cellular cooperation in a number of physiological events, including embryogenesis and morphogenesis¹. Cell-to-cell contact has been observed in lymphoid-macrophage cell cultures activated by antigens^{2,3}; furthermore, it is recognised that proliferative responses to mitogenic lectins are dependent upon contact between cells^{4,10}. To date, the nature of the cellular associations in lymphoid-macrophage cultures activated by antigens and mitogens has not been clarified. We designed the present study to evaluate some aspects of cellular associations in lymphoid-macrophage cultures activated by a variety of antigens and the mitogenic lectin, phytohaemagglutinin (PHA). Enhanced cellular aggregation was observed in cultures activated by all agents tested. Time and temperature experiments indicate that aggregation is an active process, distinct from passive agglutination. The cellular association is sensitive to proteinase and the calcium chelator, EGTA, suggesting that both proteins and calcium are essential for the cell aggregation of activated cultures.

All experiments utilised heparinised human peripheral blood sedimented in 5% dextran. The leukocyte-rich plasma was layered onto a ficoll-hypaque gradient and centrifuged at 700g for 45 min. Separated cells were washed three times in Dulbecco's phosphate-buffered saline and resuspended at a final concentration of 6×10^5 cells ml⁻¹ in RPMI-1640 media supplemented with penicillin and streptomycin (Associated Biomedic Systems, Buffalo, New York) and 10% heat inactivated (56 °C for 30 min) single donor human AB+ sera. The appropriate mitogen or antigen was then added to the cells. The cells were placed into wells of microplates (Falcon Plastics) in 0.2 ml volumes and incubated at 37 °C in a humidified atmosphere with 5% CO₂. Cellular aggregation was determined by directly aspirating the contents of the single microplate well with either a Pasteur pipette or a tuberculin syringe fitted with a flat-tipped 19 gauge needle and counting the cell suspension on a haemocytometer. The number of free cells counted, clusters of three or more cells being excluded, was found to be inversely proportional to the amount of cellular aggregation.

In the first series of experiments we determined the degree of cellular aggregation as a measure of cellular association. Results of a typical experiment, plotted in Fig. 1, indicate that purified PHA produces a significant degree of cellular aggregation (as determined by a decrease in the number of free cells). Additional data (not shown) demonstrate that the total number of cells in culture increases during the culture period, indicating that the observed decrease in the number of free cells is not due to a decrease in the total number of cells. As shown in Fig. 1, control cultures also exhibit some degree of cellular aggregation. It should be noted that a purified form of PHA, HA 16/17 (Burroughs-Wellcome), was used in these studies. HA 16/17 is known to have markedly reduced haemagglutinating activity compared to the crude extracts of the mitogen⁵. In additional experiments (data not shown), aggregation was observed with all mitogenic and antigenic agents tested. These included purified protein

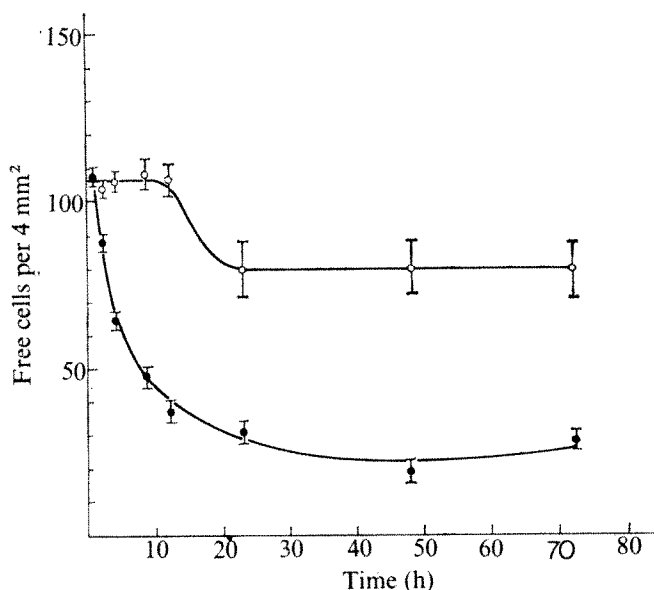


Fig. 1 Cellular aggregation in control (○) and PHA-HA 16/17 (●) treated cultures of Fcoll-Hypaque separated mononuclear cells from human peripheral blood. PHA-HA 16/17 was added to the cultures at a dilution of 1:50. Time after addition of PHA is plotted against number of cells not in aggregates. Each point represents the mean of replicate samples ± 1 s.d.

derivative of human tuberculin (PPD) (Ministry of Agriculture, Fisheries and Food, Surrey, UK), streptokinase-streptodornase Varidase, (SK/SD) (Lederle Labs), the calcium ionophore A23187 (Eli Lilly, Indianapolis, Indiana), and mixed leukocyte culture using allogeneic lymphocytes. Maximum aggregation was seen on day 6 for PPD, SK/SD, and mixed leukocyte cultures, on day 2 for PHA-HA 16/17, and on day 3 for A23187. It would appear that it is the action of the various activators (that is, the actual activation of the lymphocytes) rather than the presence of the activators that is responsible for the cellular aggregation. If the presence of the mitogens or antigens were sufficient for cellular aggregation, then one would expect to see aggregation earlier than 2 h of culture and perhaps in cultures that have the mitogens and antigens present but are maintained at 4 °C. In neither of these situations did we find aggregate formation.

Several lines of investigation suggest that aggregate formation is an active process which has several requirements: (1) a specific time period, (2) a metabolically active cell, and (3) the presence of calcium. First, aggregate formation initially appears at 2 h of culture with a progressive increase in aggregate formation up to 24 h (Fig. 1). Observation of this aggregate formation process through an inverted microscope revealed a continual increase in first the number and then the size of aggregates during the 24-h period. Second, in no instance do aggregates form when cultures are kept at 4 °C during the first 24 h of culture, regardless of whether the activating agent is

Table 1 Time- and dose-dependent reversal of PHA-HA 16/17-induced cell aggregation by the calcium chelator EGTA

	Free cells per 4 mm ² * (mean ± 1 s.e.)
No HA 16/17	228 \pm 8
HA 16/17†	36 \pm 2
HA 16/17 + 1 mM EGTA‡	74 \pm 11
HA 16/17 + 5 mM EGTA‡	92 \pm 8
HA 16/17 + 10 mM EGTA‡	121 \pm 11
HA 16/17 + 10 mM EGTA§	160 \pm 28

* Free cells counted after all cells were in culture for 48 h.

† HA 16/17 added at a dilution of 1:50 at the start of culture.

‡ EGTA added 1 h before counting of free cells.

§ EGTA added 2 h before counting of free cells.

PHA, A23187, PPD, or SK/SD. Third, in experiments in which the calcium chelator, EGTA (1–10 mM), is added to cultures at the same time as PHA, the EGTA completely prevents both aggregate formation and DNA synthesis. Thus, this group of experiments suggests that aggregation is distinct from passive mitogen or antigen-induced agglutination.

We next studied the structural basis for aggregate formation by testing the ability of certain agents to dissociate the cell aggregates found in stimulated lymphocyte cultures. Previous work by Moscona and colleagues demonstrated a role for calcium and trypsin sensitive

Table 2 Reversal of SK/SD-induced cell aggregation by EGTA

	Free cells per 4 mm ² * (mean ± 1 s.e.)
No SK/SD	262 ± 12
SK/SD†	63 ± 2
SK/SD + 10 mM EGTA‡	116 ± 6
SK/SD + 10 mM EGTA§	193 ± 24

* Free cells counted after all cells were in culture for 6 d.

† SK/SD added at a dilution of 1:2,000 at the start of culture.

‡ EGTA added 1 h before counting of free cells.

§ EGTA added 2 h before counting of free cells.

macromolecules in the maintenance of aggregates of neural retinal cells⁶. The calcium-specific chelator⁷, EGTA, dissociates aggregated cells in a time- and dose-dependent manner. Table 1 shows the EGTA dissociation of PHA-stimulated cells. Table 2 shows the EGTA dissociation of SK/SD-stimulated cells. Cells aggregated by another mitogen (A23187) and two additional antigens (PPD and allogeneic cells) show similar dissociation with the EGTA. To test whether the EGTA-induced dissociations are due to toxicity or to some nonspecific phenomena, we added CaCl₂ to the EGTA-containing cultures. The dissociation was completely reversed (Table 3), supporting the contention that the EGTA effects are due to calcium chelation. In addition, trypan blue dye exclusion tests indicated that the cells were completely viable. It seemed reasonable, however, that other divalent cations could also be involved in aggregate formation. To test this hypothesis, we incubated cells with EDTA, a chelator with less specificity than EGTA. We observed no additional dissociation from that seen with EGTA alone or from EGTA and EDTA together (data not shown), suggesting that calcium is the major ion necessary for cellular aggregation.

In addition, it seemed possible that the EGTA effects may not be directed at the calcium involved in cell-to-cell binding, but instead on the membrane calcium that is required for maintenance of membrane topography. Cooling lymphocytes to 4 °C is known to significantly reduce ligand-induced movement of surface macromolecules ("cap formation")^{8,9}. Thus, we designed temperature experiments to evaluate the possibility that EGTA directly affects movement of surface macromolecules. Results indicate that cells at 4 °C dissociate with EGTA with only a slight reduction in rate (Table 4), suggesting that EGTA-induced movements in the cell membrane are not the

Table 3 Reversal of EGTA-induced dissociation of PHA-HA 16/17 aggregates by calcium chloride

	Free cells per 4 mm ² * (mean ± 1 s.e.)
HA 16/17†	19 ± 3
HA 16/17 + 10 mM EGTA‡	49 ± 5
HA 16/17 + 10 mM EGTA + 4 mM CaCl ₂ §	14 ± 2

* Free cells counted after all cells were in culture 48 h.

† HA 16/17 added at a dilution of 1:50 at the start of culture.

‡ EGTA added 2 h before counting of free cells.

§ CaCl₂ added 30 min before counting of free cells.

Table 4 Effect of temperature on EGTA dissociation of PHA-HA 16/17-induced cellular aggregation

Duration of incubation (min)	Control	10 mM EGTA
	Cells maintained at 37 °C	
30	14 ± 2*	27 ± 1*
60	15 ± 2	40 ± 2
120	19 ± 2	68 ± 2
	Cells maintained at 4 °C	
30	17 ± 1	21 ± 1
60	15 ± 1	26 ± 1
120	13 ± 0	41 ± 2

* Free cells: mean ± 1 s.e.

major reason for the experimental results.

Finally, we examined the role of proteins in cellular aggregation by adding proteinase to cell aggregates. A typical experiment illustrated in Table 5 shows that trypsin causes aggregate dissociation to a degree quite comparable to that of EGTA.

Table 5 Dissociation of PHA-HA 16/17-induced cell aggregation by trypsin

	Free cells per mm ² * (mean ± 1 s.e.)
HA 16/17†	18 ± 3
HA 16/17 + 0.50% trypsin‡	41 ± 1
HA 16/17 + 10 mM EGTA§	46 ± 2

* Free cells counted after all cells were in culture 48 h.

† HA 16/17 added at a dilution of 1:50 at the start of culture.

‡ Trypsin (1:250, Difco) added 30 min before counting of free cells.

§ EGTA added 2 h before counting of free cells.

In summary, our data suggests that the cellular aggregation produced in lymphoid cultures activated by a variety of antigens and mitogens is an active, time-, energy-, and calcium-requiring process. Trypsin sensitive molecules appear to be involved in cellular aggregation. The molecular nature of the trypsin-sensitive molecule(s) is not clear at present. Our EGTA and calcium reversal data suggest that calcium is directly involved in the binding process. In our experimental conditions, we were unable to demonstrate that other divalent cations are involved in binding. Calcium could be directly involved in the binding process in at least two ways: It is possible (1) that the calcium could be required for maintenance of the three-dimensional structure of the trypsin-sensitive macromolecules involved in cell binding, and/or (2) that calcium, because of its divalent nature, could be involved in bridge formation between cells.

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Activation of alveolar macrophage collagenase by a neutral protease secreted by the same cell

MAMMALIAN collagenase is a proteolytic enzyme which specifically cleaves native triple helical collagen at physiological pH and temperature. As with other extracellular proteases, there is increasing evidence that this enzyme can be found in an inactive, latent form which may represent a proenzyme or an enzyme-inhibitor complex¹⁻³. It has been known for some time that, like the zymogen forms of the pancreatic proteases, latent collagenase could be activated with low concentrations of trypsin²⁻³. The existence of inactive mammalian collagenase has been perplexing because this is often the major state in which the enzyme is found: it has not been known whether trypsin activation of latent collagenase has any physiological significance. Drawing parallels to the physiological activation of pancreatic chymotrypsinogen, procarboxypeptidase and proelastase by pancreatic trypsin, several investigators have suggested the existence of protease(s) that can activate latent collagenase in the physiological conditions in which collagenase functions¹⁻³. The study reported here validates this concept by demonstrating that, in a single cell type, the alveolar macrophage, secretes an inactive form of collagenase that can be activated in physiological conditions by a protease secreted at the same time by the same cell type.

Pulmonary alveolar macrophages (PAM), obtained by lung lavage of BCG-treated rabbits, were cultured at 37 °C with daily changes of Dulbecco's modified Eagle's medium (without serum) as previously described⁴. More than 99% of these cells can be shown to be macrophages by their morphology and ability to ingest latex particles⁵. Collagenase activity in the PAM culture media was quantified with a ¹⁴C-collagen fibril lysis assay using neutral-salt-extracted guinea pig ¹⁴C-collagen (40,000 c.p.m. mg⁻¹ collagen)⁶. All assays were performed in conditions in which peptide release was linear with time and concentration of enzyme. Previous studies of the reaction products of PAM collagenase have demonstrated its specificity in cleaving types I and III collagen approximately three-quarters of the distance from the N-terminus of the native collagen⁷. In normal culture conditions, 65–70% of the collagenase produced by PAM was in the active form, with the latent enzyme able to be activated with trypsin⁵.

Table 1 Relationship of the percentage of pulmonary alveolar macrophage collagenase that is in the active form to the neutral protease activity in the same culture medium

Collagenase in active form (%)	Neutral protease activity in culture medium (U ml ⁻¹)
0	0
7	0.2
25	1.8
63	3.2

The percentage of collagenase in active form was calculated from the formula: (collagenase activity in culture medium) × 100 / (collagenase activity in culture medium after activation with trypsin). The different levels of neutral protease activity present in PAM culture medium were modulated by adding BSA in concentrations up to 20 mg ml⁻¹ to the PAM cultures after 24–48 h of incubation. With BSA (20 mg ml⁻¹) all the collagenase in the PAM culture medium was in the latent form and no neutral protease activity was detectable; with no BSA added to the PAM cultures, 65–70% of the collagenase was found in the active form. In these cultures, total collagenase activity was 6–10 µg of collagen lysed per h per 10⁶ cells⁵. Intermediate values demonstrated that the relationship between the percentage collagenase in active form and units (U ml⁻¹) of neutral protease in the culture medium was linear.

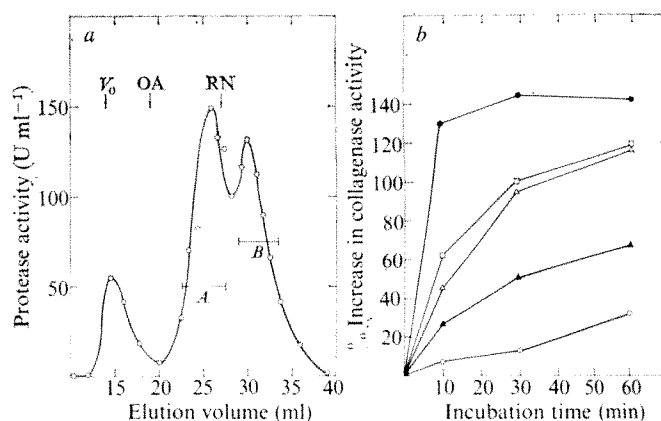


Fig. 1 Gel filtration chromatography of neutral protease activities found in culture media of rabbit alveolar macrophages and the use of these proteases in activating latent PAM collagenase. *a*, PAM culture medium collected between 24 and 48 h was subjected to ultrafiltration through a PM 30 membrane; the filtrate was concentrated with Aquacide II (Calbiochem) and chromatographed on a column (0.9 × 30 cm) of Ultragel AcA54 (LKB, 5% acrylamide–4% Agarose) with 50 mM Tris-HCl, pH 7.4, 5 mM CaCl₂, 200 mM NaCl as the eluting buffer. To determine neutral protease activity, fractions (0.5 ml) were incubated (3 h, 37 °C) in a shaking water bath with 200 µg of ¹⁴C-globin (5 × 10⁴ c.p.m. mg⁻¹), 50 mM Tris-HCl, pH 7.4, 5 mM CaCl₂ and 200 mM NaCl. After incubation, trichloroacetic acid (10%) was added (1 h, 4 °C) to precipitate the intact ¹⁴C-globin. After centrifugation (10,000g, 5 min) the supernatant containing ¹⁴C-peptides was counted in Aquasol. 1 U = digestion of µg of globin per h; this is equivalent to the activity of 15 ng of trypsin in the same assay. Ovalbumin (OA) and ribonuclease (RN) were used as molecular weight standards. Fractions eluting in region A (protease fraction A) and B (protease fraction B) were pooled and concentrated with Aquacide II. *b*, To activate latent collagenase, PAM culture medium concentrated with a PM 30 membrane was preincubated (before collagenase fibril assay) at 37 °C for 0–60 min in buffer (50 mM Tris-HCl, pH 7.4, 5 mM CaCl₂, 200 mM NaCl) plus protease fraction A (▲), protease fraction B (△), PM 30 filtrate protease (concentrated PM 30 filtrate of PAM culture media) (□) or trypsin (5 µg ml⁻¹) (●). Incubation with buffer alone (○) served as a control (no added enzyme). PM 30 filtrate protease, protease fraction A and protease fraction B had no collagenase activity in the ¹⁴C-collagen fibril assay. The amount of each used for collagenase activation had approximately the same neutral protease activity in the ¹⁴C-globin assay. After preincubation, BSA (10 mg ml⁻¹) was added to inhibit the action of the neutral protease on any other proteins in the subsequent collagenase assay; in all cases, this concentration of BSA completely stopped all neutral protease activity present. In the conditions used, it was assumed that preincubation with trypsin would activate all the latent collagenase in the culture medium (6–10 µg collagen lysed per h per 10⁶ cells⁵). The percentage increase in collagenase activity due to activation by preincubation was defined as: ((collagenase activity after preincubation) – (collagenase activity without preincubation)) × 100 / (collagenase activity without preincubation). Before preincubation, 60% of the collagenase was in the latent form.

Neutral protease activity in PAM culture medium was quantified as the trichloroacetic-acid-soluble ¹⁴C-peptides release from ¹⁴C-globin purified from rabbit reticulocyte haemoglobin labelled with ¹⁴C-leucine¹⁰. The protease assay was performed in conditions in which the release of peptides was linear with time and enzyme concentration.

When preliminary studies demonstrated that PAM cultures with large quantities of neutral protease also had large quantities of active collagenase (and comparably less latent collagenase), methods were developed to evaluate a possible relationship between these enzymatic activities. The addition of bovine serum albumin (BSA) to the PAM cultures was found to be a useful means of varying the relative amounts of neutral protease activity available in the culture media, for the BSA acted as a competitive inhibitor of the action of neutral protease on other proteins while not significantly altering collagenase secretion. When an excess of BSA was added, little protease activity and relatively less

active collagenase was found (Table 1). Alternatively, when no BSA was added, there was more protease activity and relatively more collagenase was in the active form. Thus, there seemed to be a direct relationship between the percentage of the total secreted PAM collagenase that was active and the relative amount of neutral protease activity in the same culture media.

Since the bulk of PAM neutral activity in PAM culture medium was less than 30,000 daltons, partial purification was achieved by ultrafiltration through an Amicon PM 30 membrane and concentration of the filtrate. When this material was chromatographed (Fig. 1a), neutral protease activity was found in three regions; a minor peak in the void volume (>60,000 daltons) and two major peaks, one at 20,000 daltons (protease fraction A) and one at 11,000 daltons (protease fraction B). Peak activities of protease fractions A and B were concentrated and neither were found to have active or latent collagenase activity. The activity of these fractions against the ^{14}C -globin substrate was inhibited by 10 mM ethylenediaminetetraacetate, BSA (10 mg ml $^{-1}$) or 5% serum but not by 2 mM phenylmethylsulfonylfluoride, α_1 -antitrypsin (100 $\mu\text{g ml}^{-1}$) or 10 mM N-ethylmaleimide.

One possible physiological role of these protease fractions is demonstrated in Fig. 1b, which shows that latent collagenase from PAM culture medium was activated in physiological conditions by preincubation (before the collagenase assay) with the neutral protease(s) secreted by the same cells. Preincubation with protease fraction A increased collagenase activity by 65% while protease fraction B increased collagenase activity by 120% (which was 90% that achieved with trypsin). The activation of latent collagenase by protease fraction B was the same as that achieved with partially purified (PM 30 filtrate) protease. Evidence that the neutral protease(s) truly activated latent collagenase (as opposed to appearing to "activate" by directly attacking the ^{14}C -collagen substrate in the collagenase assay) came from two observations: (1) neither fraction A or B had collagenase activity in the fibril assay, and (2) at the end of preincubation, sufficient BSA was added to inhibit all neutral protease activity. Further evidence for the role of neutral protease(s) in activating latent collagenase came from the observation that while preincubation at 37 °C for 1 h with buffer alone caused a 30% increase in collagenase activity, this phenomena (so called "autoactivation") was obviated by including BSA with the buffer.

The secretion of degradation enzymes in an initial precursor form is well documented¹¹. It is, however, often difficult to distinguish a true zymogen, in which a portion of the precursor enzyme must be removed to activate the enzyme, from an enzyme-inhibitor complex, from which the inhibitor must be removed or inactivated to activate the enzyme. For collagenase, there is argument for both possibilities^{1-8, 12-14}. For the PAM collagenase, gel filtration of completely latent collagenase demonstrates the bulk of activatable collagenase chromatographs at approximately 49,000 daltons (Fig. 2). When this partially purified latent collagenase was activated with protease B before chromatography it was found at an apparent molecular weight of 31,000. Thus, there is direct evidence of the conversion of a latent collagenase to an active collagenase of lower molecular weight by a neutral protease secreted by the same cell. There is no possibility that the 49,000-dalton form of the enzyme is a collagenase-collagen complex, since the rabbit PAM does not synthesise collagen (unpublished observations of N. A. Elson and R.G.C.). These data, however, do not answer further the question of whether the latent collagenase is a true zymogen or an enzyme-inhibitor complex.

Activators of latent collagenase have been suggested before: for example, tadpole culture fluid activation of tad-

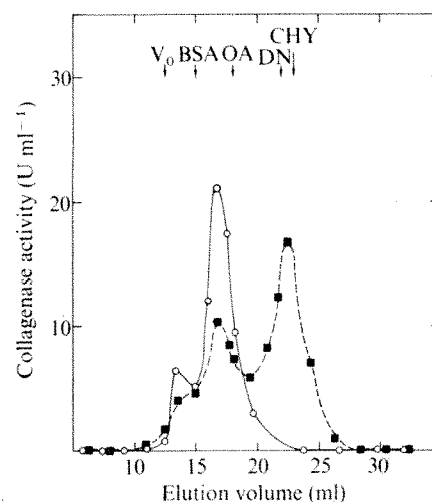


Fig. 2 Gel filtration chromatography of latent and active rabbit PAM collagenase. PAM were cultured in medium containing BSA (10 mg ml $^{-1}$). The medium was collected after 0-24 h of incubation and concentrated by Amicon PM 30 membrane ultrafiltration. The resulting material contained no neutral protease activity and 100% of the collagenase was in the latent form. Chromatography was performed on Ultragel AcA54 (LKB, 5% acrylamide-4% Agarose) eluted with 50 mM Tris-HCl, pH 7.4, 5 mM CaCl $_2$, 200 mM NaCl. Fractions (0.5 ml) were collected and assayed for collagenase activity using the ^{14}C -collagen fibril assay after trypsin activation (○) (50 μl samples of each fraction were incubated with trypsin (10 $\mu\text{g ml}^{-1}$) for 20 min at 23 °C followed by incubation with soybean trypsin inhibitor (50 $\mu\text{g ml}^{-1}$) for 10 min). In the ^{14}C -collagen fibril assay, 1 U = 1 μg of collagen lysed per h. Separate chromatography of molecular weight standards (BSA), ovalbumin (OA), desoxyribonuclease I (DN) and chymotrypsinogen A (CHY) were used to estimate the major peak of latent collagenase (fractions 16-17) at 49,000 daltons. When the PAM medium was preincubated (37 °C, 45 min) with 120 U of protease B (■) before chromatography, the major peak of collagenase activity had a molecular weight of approximately 31,000. The collagenase activity at the void volume (fractions 13-14) probably represents aggregated enzyme¹⁵; rechromatography of this larger molecular weight form in a higher ionic strength buffer (1.2 M KCl compared with 0.2 M NaCl) caused part of the collagenase activity in this large molecular weight region to shift to the major peak of collagenase activity.

pole tail collagenase¹; human rheumatoid synovial fluid activation of human granulocyte collagenase⁶; human microbial plaque activation of human gingival collagenase⁸; and mouse liver cathepsin, hog pancreatic kallikrein or human serum plasmin activation of mouse bone collagenase². Our data suggest that at least for the alveolar macrophage, the same cell type secretes collagenase in a latent form, as well as protease(s) which activate it. Both (collagenase and neutral protease) activities are demonstrable in physiological conditions and there is a correlation between protease activity and relative collagenase activation. This finding helps to explain the phenomena of collagenase "autoactivation" whereby unpurified preparations of collagenase can be activated by incubation at 37 °C, a finding that is often exaggerated when collagenase-containing culture media are concentrated. Most importantly, this demonstration of interactions between protease and latent collagenase gives a physiological explanation for *in vitro* trypsin activation of latent collagenase and suggests important control points in the regulation of collagen degradation.

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Vasopressin-induced redistribution of binding sites for concanavalin A at the surface of epithelial cells from urinary bladder

VASOPRESSIN promotes water movement across the epithelium of the distal portion of mammalian renal tubule and amphibian urinary bladder^{1,2}. The hormone also elicits a general, non-selective increase in the transcellular permeation of lipophilic solutes³ and a decrease in the discrimination between straight- and branched-chain isomers^{4,5}. Such changes in the permeability of amphibian urinary bladder are limited to the apical membrane of those epithelial cells rich in intracellular secretion granules^{6,7}. These granules, some of which may correspond to lysosomes⁸, are observed to migrate to the mucosal membrane of toad bladders treated with oxytocin or cyclic AMP⁹. Moreover, treatment with vasopressin provokes the extracellular release of acid hydrolases at the apical surface of cells from bullfrog bladder¹⁰. Additional evidence shows that repression of such hydrolase activity at the external surface reduces the hormone-induced water flow observed across the intact epithelium⁷. My present investigation extends these observations by providing direct evidence in epithelial cells from bullfrog bladder for vasopressin-induced rearrangement into clusters of binding sites for concanavalin A (con A) which are otherwise randomly disposed at the surface of control cells. This hormone effect is inhibited by prior incubation of cells with the microtubule-disrupting drug, colchicine. In addition, colchicine treatment antagonises the enhancement of extracellular hydrolase release and transepithelial water flow attributable to vasopressin action.

Epithelial cells were isolated from the urinary bladders of *Rana catesbeiana* (Mogul-Ed, Oshkosh, Wisconsin) by procedures described previously⁷. The cells were maintained at 22 °C and suspended in Ringer solution constituted as follows: 104.5 mM NaCl, 2.7 mM KCl, 0.5 mM CaCl₂, 0.6 mM MgSO₄, and buffered at pH 7.4 with 2.125 mM Na₂HPO₄/0.375 mM NaH₂PO₄. Aliquots (2 ml; approximately 3 × 10⁶ cells) were incubated in the presence or absence of test reagents and appropriate control compounds for selected times in a Dubnoff shaking incubator. Epithelial cells in the several experiments were exposed to arginine vasopressin (AVP), chlorobutanol, colchicine, α-methyl-D-mannoside (all from Sigma), con A (Calbiochem), or combinations of these agents. Colchicine was converted to biologically inactive lumicolchicines by irradiation with ultraviolet light as described by Wilson and Friedkin¹¹. Cell viability was evaluated by the method of dye exclusion¹². At least 95% of all cells used here excluded the dye, Trypan blue, at a final concentration of 0.05% during 10 min incubation in Ringer solution at 22 °C.

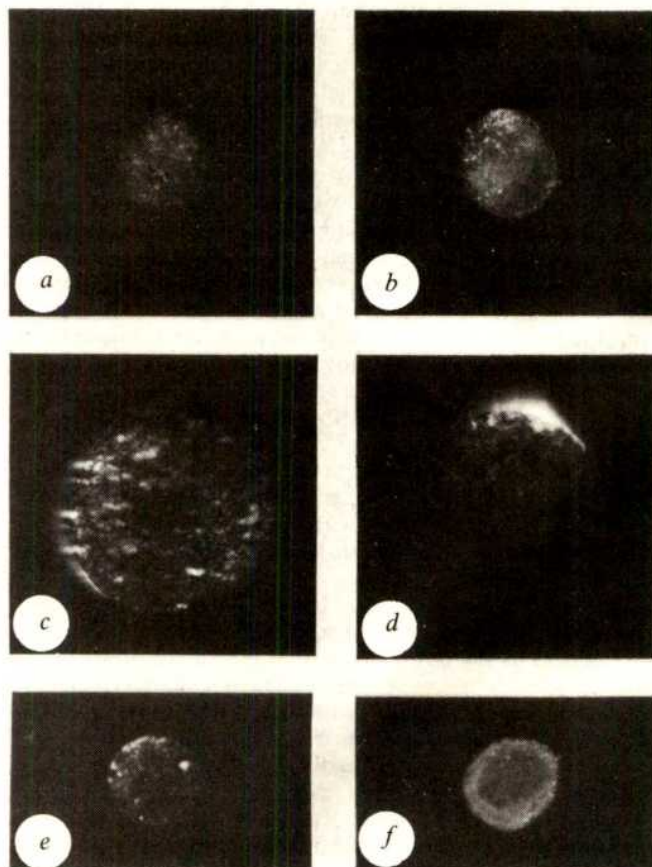
Previous studies have shown that epithelial cells isolated from bullfrog urinary bladder possess specific binding sites for fluorescein-labelled con A (F-con A). Cells bind the lectin maximally at a concentration of 50 μg F-con A ml⁻¹

when incubated for 3 min at 22 °C (ref. 13). These conditions were used to evaluate, by dark-field ultraviolet-fluorescence microscopy, F-con A binding to cells that had been incubated with or without 10 mU AVP ml⁻¹ for 15 min.

F-con A was distributed in apparently random fashion over the surface of the majority of cells treated only with hormone vehicle, 5.6 × 10⁻¹¹ M chlorobutanol. A representative example is presented in Fig. 1a. Diffuse clusters of label were however, occasionally present at the surface of control cells (Fig. 1c). Addition of AVP to cells for 15 min evoked a marked concentration of fluorescence at one pole of the cell surface in approximately 45% of all isolated cells observed (Fig. 1b, d). Moreover, many of those cells treated with hormone tended to bind together, with aggregates of F-con A evident at the points of cell contact. As in previous experiments with epithelial cells from urinary bladder¹³, binding of F-con A at the cell surface could be blocked by the addition of 0.1 M α-methyl-D-mannoside or excess unlabelled Con A during the incubation of cells with F-con A (not shown).

In view of recent reports that indicate that the microtubule-disrupting drug, colchicine, counteracts the hydrosmotic effect of vasopressin in toad urinary bladder¹⁴, the

Fig. 1 Dark-field fluorescence micrographs of F-con A binding to epithelial cells from bullfrog urinary bladder. Cells were incubated in the absence (Fig. 1a, ×400; Fig. 1c, ×1,000) or presence (Fig. 1b, ×400; Fig. 1d, ×1,000) of 10 mU AVP ml⁻¹ for 15 min as described in the text. Additional cells were incubated for 3 h with 2 × 10⁻⁵ M lumicolchicine (Fig. 1e, ×400) or 2 × 10⁻⁶ M colchicine (Fig. 1f, ×400) before exposure to hormone for 15 min. Photomicrographs were obtained with a Leitz ortholux microscope used in conjunction with a xenon light source, XBO 150. The exciting filter combination consisted of a BG 38 heat barrier filter, a Leitz 480 nm filter and a Leitz KP 490 interference filter; a K 510 nm filter served as the barrier filter. Photographs were taken on Kodak Tri-X pan film (ASA 400). Approximately 250 cells in each treatment group were observed in each of four to six independent experiments.



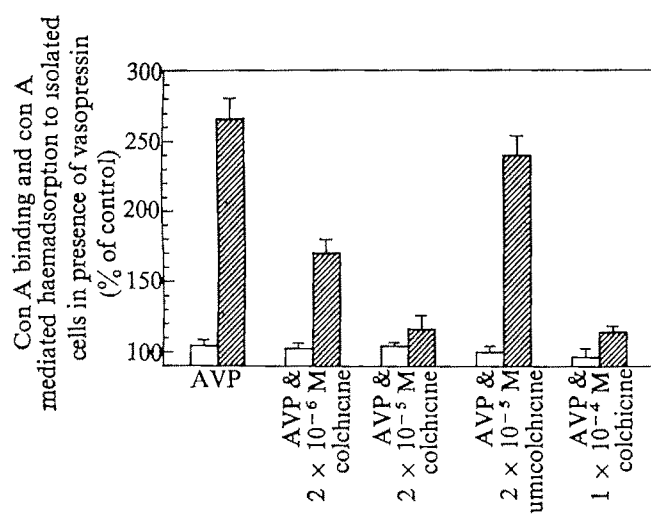


Fig. 2 Effects of prior incubation with various concentrations of colchicine or lumicolchicine (3 h) on F-con A binding (clear bars) and con A-mediated haemadsorption (shaded bars) to epithelial cells in the presence and absence of 10 mU AVP per ml. In methods described previously¹³, maximal F-con A binding to cells was detected at 50 μ g lectin per ml (3 min, 22 °C). With a spectrophotofluorometric value of 6 net fluorescence (arbitrary units) equivalent to approximately 0.10 μ g of con A, lectin binding to the present cells in the absence of added reagents averaged 12.2 ± 0.7 net fluorescence per mg cell protein. Adsorption of isologous erythrocytes to bladder cells was determined from measurements of haemoglobin absorbance (418 nm) associated with filtered/solubilised epithelial cells previously exposed to con A as described before¹³. The basal level of con A-mediated haemadsorption, expressed as absorbance per mg epithelial cell protein, averaged 0.024 ± 0.004 . Treatment of epithelial cells with colchicines alone did not significantly influence these baseline levels of lectin binding or lectin-mediated haemadsorption to cells. The significance of differences between treatment and control preparations was estimated by paired analyses with the Student's *t* test. All experimental values were calculated as mean \pm s.e.m. and are presented as percentage of appropriate controls.

surface distribution of F-con A was examined in the presence and absence of hormone following 3 h prior incubation with colchicine or its biologically inactive derivative, lumicolchicine. As in controls not treated with hormone or drugs, distribution of lectin-binding sites was random at the surfaces of cells exposed only to colchicine or to lumicolchicine (not shown). Addition of hormone to cells incubated with lumicolchicine evoked the characteristic polar redistribution of con A binding sites (Fig. 1e). In contrast, the orientation of label remained diffuse at the surfaces of colchicine-treated cells despite the presence of AVP (Fig. 1f).

A quantitative estimate of lectin-mediated cellular agglutinability in the presence and absence of AVP was obtained by a haemadsorption method previously described¹³. In confirmation of earlier observations¹³, treatment of epithelial cells of bullfrog bladder for 15 min with AVP elicited a marked increment in con A-mediated haemadsorption ($P < 0.001$), but not F-con A binding ($P > 0.90$), as compared to the corresponding values for control cells not exposed to hormone (Fig. 2). This enhancement of the lectin-mediated cellular agglutinability by hormone treatment correlates with the AVP-induced redistribution of F-con A binding sites at the cell surface as described above.

As shown in Fig. 2, epithelial cells treated with AVP after incubation with lumicolchicine continued to show a significant enhancement of con A-mediated haemadsorption ($P < 0.001$), but not lectin binding ($P > 0.90$), as compared to corresponding cells not exposed to hormone. However, treatment of bladder cells with colchicine before addition of AVP evoked a substantial reduction in the expected level of lectin-mediated haemadsorption ($P > 0.05$), but not lectin binding ($P > 0.90$), to cells as compared to that of appropriate

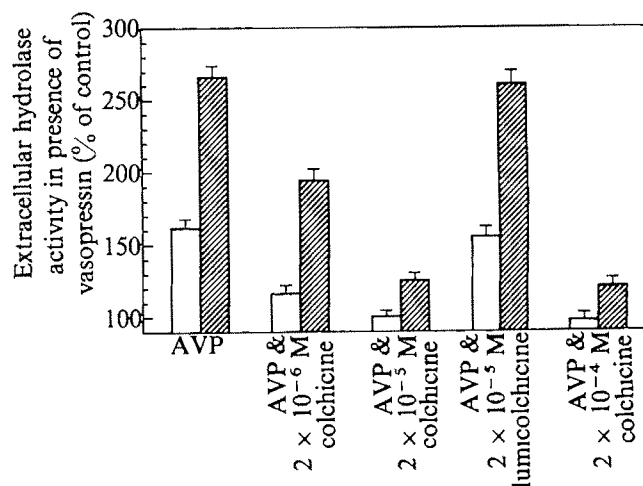
control cells (Fig. 2). The decline in hormone responsiveness was proportionate to the medium concentration of colchicine (Fig. 2).

The influence of colchicine on the AVP-induced release of acid hydrolases from isolated epithelial cells^{7,10} was investigated in further experiments shown in Fig. 3. Treatment of cells with 10 mU AVP per ml alone for 7.5 min enhanced the extracellular activities of cathepsin B1 and acid phosphatase to 266% and 162% of the levels associated with control cells, respectively. Prior treatment of cells with 2×10^{-5} M lumicolchicine for 3 h before exposure to AVP elicited no significant alteration in the hormone-dependent elevation of extracellular hydrolase activities as compared to control cells not treated with AVP (both at $P > 0.90$). But these normal increments in extracellular enzyme activities were markedly reduced among epithelial cells incubated with colchicine at concentrations ranging from 2×10^{-6} to 1×10^{-4} M for 3 h before exposure to AVP.

In view of the above evidence for inhibition of actions of AVP in isolated cells by colchicine, analyses were undertaken of the influence of the drug on hormone-enhanced water permeation across intact epithelium derived from bullfrog. As shown in Fig. 4, treatment of intact bladder with AVP for 1 h elicited an average increment of 600% in water permeability as compared to that of control tissues after prior incubation with lumicolchicine at concentrations ranging from 2×10^{-7} to 1×10^{-4} M (all at $P < 0.001$). However, treatment of bladders with colchicine before addition of hormone markedly reduced the expected increase in water movement attributable to hormone. The maximal inhibition of AVP-induced water flow was observed at a colchicine concentration of approximately 2×10^{-5} M.

The results of these studies show that AVP treatment of epithelial cells isolated from urinary bladder provokes a marked rearrangement of membrane binding sites for con A at the external surface. This response to hormone occurs in the absence of significant alterations in specific con A binding to cells. Moreover, AVP-induced redistribution of

Fig. 3 Effects of prior incubation with various concentrations of colchicine or lumicolchicine (3 h) on extracellular release of acid phosphatase (clear bars) and cathepsin B1 (shaded bars) from epithelial cells treated with and without 10 mU AVP per ml. The activities of cathepsin B1 (EC 3.4.22.1) and acid phosphatase (EC 3.1.3.2) were determined on aliquots of particle-free supernatant fractions collected from the incubation media of isolated cells after 7.5 min treatment with or without hormone as described previously^{7,10}. With extracellular enzyme activities expressed as pmol substrate utilised per min per mg cell protein, basal activities of cathepsin and phosphatase averaged 38 ± 3 and $1,030 \pm 24$, respectively. Treatment of cells with colchicines alone did not significantly alter these control values for extracellular enzyme activity. All experimental determinations are presented as percentage of appropriate controls.



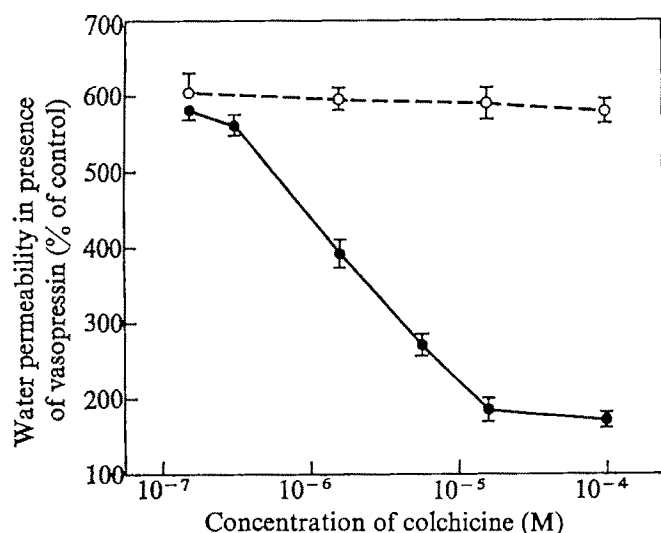


Fig. 4 Effects of prior incubation with various concentrations of colchicine (●) or lumicolchicine (○) on AVP-induced water permeation across bullfrog urinary bladder. Colchicines were present for 3 h in mucosal and serosal media before serosal addition of 10 mU AVP per ml. Radioactive tracer techniques were used to study the movement of water in the mucosal to serosal direction across bladders mounted as flat sheets between two Lucite chambers as described previously³. Permeability measurements were made during the 60-min period following addition of AVP or hormone vehicle. Basal water permeation was corrected for the presence of unstirred layers (170 μ m) as before^{3,7} and averaged $1,200 \pm 100 \times 10^7$ cm s⁻¹. Treatment of bladders with colchicines alone did not significantly influence the water permeability coefficient as compared to paired tissues not exposed to colchicines. All experimental values are presented as a percentage of appropriate paired controls.

lectin-binding sites, as indicated by fluorescence microscopy, may contribute to the enhancement of con A-mediated cellular agglutination¹³. Clustering of lectin-binding sites and the resultant increase in agglutinability of transformed cells has been considered to be a consequence of increased lateral movement of antigen-binding components in the plane of the membrane^{15,16}.

The findings likewise correlate with other evidence for redistribution of intramembranous particles associated with apical membrane of urinary bladder after exposure to neurohypophyseal hormones^{17,18}. In addition, the mobility of spin-label and fluorescent probes and the movement of non-electrolytes in the lipid phase of several cell types are known to increase in response to addition of the appropriate polypeptide hormone^{4,5,18-21}. Such increments in the motional freedom of membrane molecules attributable to hormone may contribute to the aggregation of lectin-binding sites observed in this work.

It has been shown that disruption of microtubules by colchicine treatment reduces AVP-induced water flow across amphibian urinary bladder¹⁴, a finding verified here. This drug effect is accompanied by a marked reduction in lectin-mediated cellular agglutination and in extracellular hydrolase release which normally occurs in response to AVP exposure. Microscopic observation of the distribution of F-con A at the external surface demonstrates that AVP-induced movement of lectin-binding sites is inhibited in cells treated with colchicine, but not lumicolchicine, before exposure to hormone. Thus, it seems that cellular components sensitive to colchicine are essential to the AVP-induced redistribution of lectin receptors at the bladder cell surface. This seems inconsistent with the conclusion that colchicine-sensitive structures constrain the movement of recognition sites for lectins²² and antibodies²³ at the external surfaces of leukocytes. Other studies indicate however that, under some circumstances (such as phagocytosis), colchicine-binding proteins not only serve to anchor surface

elements but are also required for their directed movement²⁴⁻²⁶. The specific displacement of con A binding sites from their random distribution into clusters after AVP exposure, as shown here, likewise seems to require coupling with intracellular elements sensitive to disruption by colchicine.

As suggested from the results of previous studies^{7,8,18,14}, microtubules may participate in the action of AVP on water flow across urinary bladder through their involvement in the mechanism of release of granular enzymes at the apical surface of epithelial cells. Clearly, additional work is necessary to determine the functional significance of this early vasopressin effect on cellular hydrolase distribution. The present report provides additional support for the view that ensuing hormone-induced alterations in the orientation of membrane proteins and in the degree of order or flow of membrane lipids may underlie changes in epithelial transport functions due to vasopressin treatment.

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Antigen-specific helper factors in rabbit lack both V and C region Ig determinants

THE nature and genetic origin of the T lymphocyte antigen-recognition system is an intriguing and compelling immunological problem. T cells differ from B cells in not synthesising classical antibody and in not expressing a readily detectable immunoglobulin (Ig) as their surface receptor for antigen¹. It has recently been found, however, that in some cases T cells and serum antibody carry the same idiotypic determinants²⁻⁴, inferring identity between the binding sites, that is the variable regions, of the T cell antigen receptor and immunoglobulin. It has also recently been discovered that T cells can produce antigen-specific molecules or factors which carry determinants of the major histocompatibility complex (MHC) in addition to a binding site for antigen⁵⁻⁸. The existence of the specific T cell factors raises the possibility of variable (V) region genes

separate from those contributing to antibody and B cell receptors, and perhaps located in the MHC¹. In order to resolve these possibilities we have attempted to produce antigen-specific T cell factors in the rabbit. The rabbit is the ideal animal for such a study, since it uniquely carries allotypic markers in the V region of its immunoglobulins (see reviews in refs 10 and 11). The sharing of such a V-region allotypic marker by both Ig and the specific T cell factor would establish a common genetic basis for antigen recognition in the two molecules. The results below show, however, that the Ig V region allotypes determined by the *a* locus and indeed other Ig markers are not present on the specific T-cell helper factor in the rabbit and imply the existence of separate V genes for antibody and T cell factors.

The antigen-specific helper factor was obtained from the peripheral blood lymphocytes of rabbits immunised with sheep erythrocytes (SRBC). In testing the factors, advantage was taken of the ability of the specific helper factors to collaborate with B cells across a species barrier. It has been previously shown that the mouse T cell factor will interact with and trigger human lymphocytes in culture^{12,13}. In the experiments described here, the rabbit factor was tested for its ability to cooperate with mouse B cells in a response to SRBC when transferred into irradiated mice. In detail, rabbits were inoculated intravenously with 0.1 ml packed SRBC. Four days later, heparinised blood was taken and erythrocytes removed by sedimenting at 1g in 3% gelatin at 37 °C. Peripheral blood lymphocytes, without further separation, were washed and suspended at a density of 5×10^6 cells ml⁻¹ in RPMI 1640 (Microbiological Associates) containing glutamine and 10% foetal calf serum (Gibco). The cells were incubated under CO₂ at 37 °C in 50 mm Petri dishes for 6–8 h, together with antigen, that is, 0.1 ml 0.1% SRBC per ml of culture. At the end of incubation cell viability was usually greater than 80%. The cells were removed by centrifugation and the culture supernatant was used as the source of the rabbit factor. Its activity was assayed by mixing together with mouse (C57BL/10ScSn) bone marrow cells and antigen (SRBC) and transferring into irradiated (700 R) syngeneic mouse recipients (2×10^7 bone marrow cells, 0.1 ml 10% SRBC per recipient). The plaque forming cell (PFC) response in the recipients was measured 8–10 d after transfer and compared with that in appropriate control groups. The rabbits used were homozygous and heterozygous at the *a* and *b* loci. The *a* locus determines allotypes of the V region of the heavy chains, while the *b* locus allotypes are present on the κ light chains (see refs 10 and 11). The antiallotypic antisera were raised by a method published earlier¹⁰. Goat anti-rabbit Fc was raised against purified rabbit IgG Fc and absorbed with the Fab fragment. All the antisera were used as immunoadsorbents by coupling the 18% Na₂SO₄ precipitated fraction to activated Sepharose¹⁴.

The results in Table 1 show that primed rabbit peripheral blood cells on incubation with antigen can release a factor which cooperates with mouse bone marrow cells. In the presence of the rabbit factor, the mouse B cells proceed to an IgM anti-SRBC response which would otherwise require the presence of mouse T cells (Table 1). The rabbit factor is specific in its effect and will not cooperate in a response to horse RBC. Further, the factor is absorbed out by packed

Table 2 Effect of anti-Ig adsorbents on rabbit helper factor for SRBC

Immunoadsorbent	PFC†
—	3,200 ± 365
Anti-a†	2,450 ± 280
Anti-b†	4,900 ± 390
Anti-Fc	3,000 ± 323
Control*	200 ± 26

* Bone marrow cells alone.

† The rabbit used in this experiment was homozygous *a*1 and *b*4.

‡ Geometric means ± s.d.

SRBC (not shown in Table 1), presumably by virtue of possessing an antigen binding site. We have not yet sought proof that the rabbit factor is a product of rabbit T cells, although the obvious similarity with the antigen-specific mouse factor makes this very likely. It is also noteworthy that not all rabbits tested produced the factor; the reasons for this may be technical, but are worth closer scrutiny. The ability of the rabbit factor to act in the mouse is very reminiscent of the xenogeneic reaction of mouse T cell factors with human lymphocytes^{12,13} and again underlines that the restrictions on cellular interactions¹⁵ do not apply to the helper factors.

In order to detect Ig V and C region determinants on the rabbit factor, the supernatants of the rabbit cell cultures were passed through Sepharose immunoadsorbents carrying anti-*a*, anti-*b* or anti-Fc antibodies. The ability of these adsorbents to remove appropriate rabbit Ig was established. However, the results, of which Table 2 is typical, showed that none of the anti-Ig adsorbents tested was able to bind the rabbit helper factor, since no reduction in helper factor activity was observed after passage through the adsorbents. Partial removal of the factor by the immunoadsorbents would have been detectable if in excess of 50%. Hence the rabbit factors, though able to bind antigen, apparently do not carry the V region *a*-allotypes, the κ light chain *b* allotypes or determinants of the constant region of the γ chain. It may be noted that the expression of the *a*-allotypes on rabbit heavy chains is not dependent on the presence of a light chain.

These results indicate that the helper activity released by rabbit lymphocytes is not due to classical serum antibody. It has already been shown in the mouse that T cell derived antigen-specific helper and suppressor factors lack the determinants of the constant regions of both heavy and light chains^{4,7}. The present work shows that, in addition, the analogous molecules in the rabbit lack the allotypic markers associated with the V regions of the majority of circulating Ig molecules. We would propose that this result also holds true for the T cell surface receptor for antigen, since it is very unlikely that the V region of the T cell factor would be different from that of the T cell receptor. It should be noted that a small proportion (10%) of antibody molecules in the rabbit also lack the *a* locus allotypes (see refs 10, 11) and we cannot rule out the possibility that the factors share V regions with this minority Ig population. Taken together with molecular studies of the antigen-specific helper factor in the mouse^{3,9,12}, however, these findings would tend to support the existence of an Ig-independent V-gene pool for T cell recognition. Since the mouse factor is at least partially coded by genes in the I region of the mouse H-2, the T cell V gene pool might also be expected to be associated with the major histocompatibility complex⁹.

Our result seems to be at odds with those of other workers who have demonstrated the presence of antibody idiotypes on T cells specific for histocompatibility antigens¹, phosphorylcholine¹, and streptococcal carbohydrate². Genetic linkage for some of these shared idiotypes to Ig allotypes has been established in the mouse¹⁶, though the possibility that the idotype-bearing molecule is passively acquired from serum has not been ruled out in all cases¹⁶. It could be

Table 1 Activity of a rabbit helper factor for SRBC

Cells and factors transferred	PFC response*
Bone marrow+SRBC	180 ± 25
Bone marrow+thymocytes+SRBC	6,300 ± 520
Bone marrow+rabbit factor+SRBC	3,650 ± 390
Bone marrow+rabbit factor+HRBC†	250 ± 22

* Geometric means ± standard deviation.

† HRBC, horse red blood cells.

argued that the a allotypes, in contrast with idiotypes, are primarily a marker of the framework of the V region. The sharing of idiotype determinants by T cell receptors and antibody might then be explained by a mechanism in which Ig hypervariable regions in the form of episomes are inserted into a non-Ig T cell V framework, on the lines of a suggestion already put forward for the antibody itself¹⁷⁻¹⁹. In this case, the T cell factors, while lacking Ig V-region allotypes, could nevertheless carry Ig idiotype determinants. It remains to be established whether the T cell factors themselves carry Ig idiotypes, and this possibility is presently being investigated.

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Specific unresponsiveness to transplantation antigens induced by auto-immunisation with syngeneic, antigen-specific T lymphoblasts

AUTOIMMUNE reactions have hitherto been considered to be harmful or at best neutral for the individual. Specific autoimmune reactions in certain experimental systems may, however, have consequences of potential positive value for the individual¹. Here, advantage was taken of the fact that antigen-binding receptors of B and T lymphocytes belong to the classes of self-molecules against which autoimmunity can be inflicted²⁻⁴. Such receptors can be classified according to specific antigen-binding capacity or unique antigenic, idiotype markers⁵. In one system studied, autoanti-idiotypic antibodies were induced against rat T and B cell receptors specific for major histocompatibility antigens of the species¹.

Soluble antigen-binding receptors were purified using anti-idiotypic immunosorbents. Autoimmunisation with purified, polymerised receptors lead to production of autoanti-idiotypic immunity. This caused a selective elimination of lymphocytes carrying the relevant idiotype, antigen-binding receptors. Consequently, such autoimmune rats could be shown to be tolerant to transplantation of skin from the proper foreign donor, whereas reactivity against third party skin was left intact. No negative side-effects were noted during immunisation. Thus, autoimmunisation against the individual's receptors for a given antigen may constitute an efficient way of producing specific immune tolerance in adult, immunocompetent individuals. This approach would have obvious possible applications in clinical transplantation immunology, in treatment of allergic disorders, and might even be used when trying to regulate the course of certain autoimmune diseases. The requirement to have accessible anti-idiotypic antibodies to make the necessary immunosorbents to obtain pure receptors, is, however, a severe drawback. An approach allowing autoanti-receptor immunisation without having to rely on sophisticated or unobtainable reagents would be of great usefulness. Here, we report on a technique whereby autoanti-receptor immunity leading to specific immune unresponsiveness can be induced *in vivo* using as immunogen the individual's own antigen-specific lymphoblasts as obtained after *in vitro* immunisation.

The rationale behind the present approach reads as follows. Lymphocytes carry on their surface idiotype, antigen-binding receptors signifying their potential immune reactivity. To obtain the particular subpopulation carrying receptors with specificity for a given antigen in a pure form two steps of purification had to be used. Whereas conventional immunosorbents made up of antigen can be used to selectively purify B lymphocytes, this approach is considerably less efficient for T cells⁶. When lymphocytes respond to immunogen they frequently, however, transform into larger cells, lymphoblasts. Such blasts can be selectively purified from non-responding small lymphocytes using 1g velocity sedimentation⁷. Purified lymphoblasts carry select reactivity against the relevant antigens only, and represent a highly enriched population of cells carrying receptors with the expected antigen-binding, idiotype-positive markers^{8,9}. Such blasts could thus be considered to represent a high local concentration of "pure" idiotype receptor molecules suitable for autoimmunisation if administered in an immunogenic form. To increase the immunogenicity of such blasts when used for autoimmunisation we emulsified the cells in Freund's complete adjuvant before inoculation. The results to be presented indicate that this immunisation procedure will lead to specific autoimmunity and selective abrogation of reactivity against the relevant antigens.

The antigens used for study were the major histocompatibility complex locus antigens of the mouse and rat. T lymphoblasts were obtained from mixed leukocyte cultures using procedures already described^{10,11}. CBA/H blasts obtained after responding to DBA/2, C57BL/6 cells, or Lewis rat T blasts reacting against DA cells were used respectively. After the final 1g velocity sedimentation procedures⁷, 10⁷ blasts or small, non-responding lymphocytes from the same MLC were emulsified in Freund's complete

Table 1 Specific suppression of MLC response to DBA/2 antigens induced by autoimmunisation of CBA/H mice with autologous anti-DB A/2 T blasts

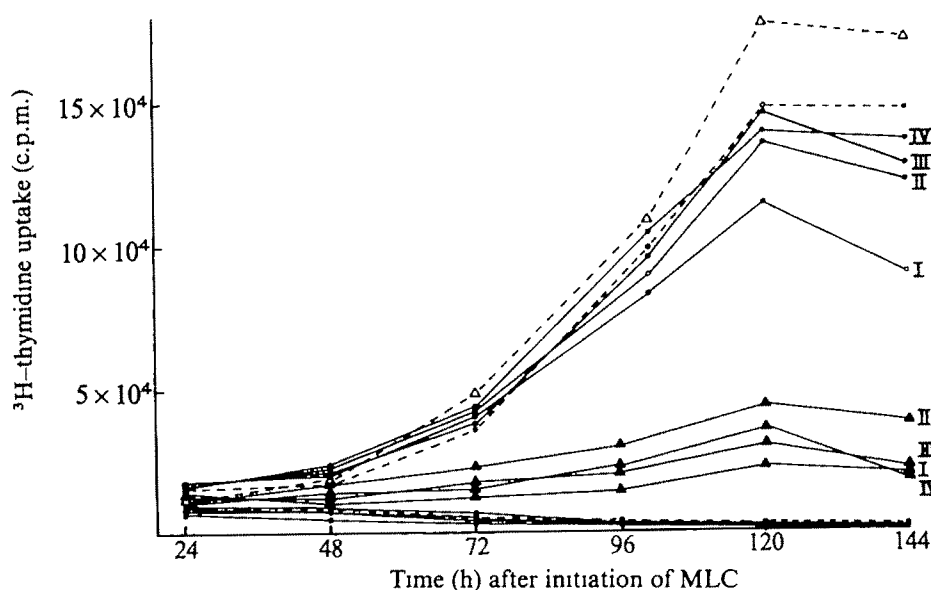
Responder* Stimulator	—	I DBA/2	ACA	—	II DBA/2	ACA	—	III DBA/2	ACA
Day 4	642±58	937±86	1823±71	667±268	1973±93	1724±182	374±103	8065±1961	2488±209
Day 6	302±57	504±143	4517±189	443±66	4429±1163	4964±1225	162±51	28251±2271	12122±580

* I: spleen cells from mice immunised with anti-DBA/2 T blasts at days 0, 60, and 120 and killed at day 130.

II: spleen cells from mice immunised with small, unresponsive CBA/H T cells from the same MLC:s as the I cells.

III: normal CBA/H spleen cells. Culture conditions as described.

Fig. 1 Demonstration of specific unresponsiveness to DA alloantigens induced in Lewis rats immunised three times with 10^7 Lewis-anti-DA T lymphoblasts from day 4 or 5 of primary MLCs. Culture conditions as previously described¹⁴. Individual experimental rats tested day 10 after last immunisation for reactivity against irradiated syngeneic or allogeneic spleen cells. —, Experimental animals; ●, Lewis syngeneic controls; ▲, Lewis against DA; ○, Lewis against; August ---, Normal Lewis.



adjuvant (DIFCO) and inoculated into one hind leg foot pad. Boosting was carried out using the same number of cells but emulsified in incomplete adjuvant. At indicated time intervals thereafter cells or sera were obtained from the animals and assayed for immune activity.

It could be shown that the present immunisation schedule leads to the induction of specific unresponsiveness against the relevant MHC antigens both in the mouse rat systems. This is depicted in a mouse combination (CBA/H spleen cells from mice inoculated with CBA/H-anti-DBA/2 T blasts displayed specific reduction of unresponsiveness against DBA/a antigens) in Table 1, and a rat combination in Fig. 1 (Lewis spleen cells from Lewis rats immunised with syngeneic blasts reactive against DA cells failed to react against DA but reacted normally against third party August cells). Preliminary kinetic data (not included) have indicated that this selective unresponsiveness may become induced within a few weeks after primary immunisation. That this unresponsiveness of the spleen cells was due to an active process stem from several observations. Using as target cells in a ^{51}Cr release assay CBA/H blasts of different specificity it could be shown that cells from autoimmunised CBA/H mice (immunised with their own anti-DBA/2 blasts) expressed specific cytolytic activity against CBA/H-

anti-DBA/2 blast as depicted in Table 2. If serum from immune animals were present during the incubation, cytotoxicity was still greater in the specific combination. Immune serum could also be shown to make normal CBA/H spleens aggressive against the CBA/H-anti-DBA/2 blasts. These data thus indicate the existence of specific immune reactivity both in cells and sera from autoimmunised mice using as targets the relevant autologous specific T lymphoblasts. The data do not show, however, whether the cytolytic effects observed are entirely due to antibody-dependent cell-mediated cytotoxicity, ADCC, or if specific anti-idiotypic killer T cells are also involved. IgG antibodies are considered to be the most efficient inducers of ADCC¹⁴. ^{125}I -labelled protein A from *Staphylococcus aureus* can be used as a marker of cell-bound IgG antibodies¹⁵. Sera from CBA/H mice immunised with CBA/H-anti-DBA/2 T blasts could be shown to react specifically with such blasts and not with other CBA/H blasts, using such ^{125}I -protein A techniques (data not shown). The present data are thus fully compatible with the view that the mice inoculated with their "own" purified T blasts with specificity for a given MHC set of antigens can indeed produce auto-anti-idiotypic antibodies.

Further support for this statement came from the analysis of the reactivity of such anti-lymphoblast sera as to their ability to react with alloantisera containing the relevant antigen-binding, idiotype-positive antibody molecules. It has previously been found possible to detect such anti-idiotypic reactions in the MHC systems using ordinary gel diffusion techniques using repeated applications to the gel basins^{3,12}. Here, we were able to show that the very same serum pool used for the experiments depicted in Table 2 (and also for the ^{125}I -protein A experiments) indeed contained antibodies reactive in a specific way not only with the relevant allo-antibodies (see Table 3) but also with concentrated MLC supernatants of the correct specificity. That the reactive factor in the MLC supernatant did indeed react with the relevant H-2 antigens was shown by specific absorptions to the relevant tissues.

In summary, we feel confident to make the following conclusions. It is possible to induce a state of selective unresponsiveness or hyporeactivity against a specific set of MHC antigens using autoimmunisation with the individual's own, *in vitro* educated T blasts with specificity for these antigens. We also know that at least in some individuals these immunisation procedures will lead to the production of detectable amounts of autoanti-idiotypic antibodies with specificity for relevant antigen-binding receptors. As previous experiments using purified soluble idiotype

Table 2 Demonstration of specific lytic activity of spleen cells and serum from autoimmunised CBA/H mice

Effector cells	Serum†	Target blasts, % ^{51}Cr release‡	kad	kaf	k ConA
I	5% FCS	51	16	< 0	< 0
I	2% NMS-0.1% IS	30	< 0	< 0	< 0
I	2% NMS	18	< 0	< 0	1
II	5% FCS	< 0	0	< 0	< 0
II	2% NMS+0.1% IS	47	11	6	6
II	2% NMS	< 0	0	< 0	< 0
III	5% FCS	< 0	< 0	< 0	< 0
III	2% NMS+0.1% IS	24	< 0	< 0	< 0
III	2% NMS	5	< 0	< 0	< 0

* Effector cells. I: spleen cells from CBA/H mice immunised three times with 10^7 CBA/H-anti-DBA/2 T MLC blasts, last time 10 d before testing II: spleen cells from CBA/H mice immunised three times with CBA/H small T lymphocytes from the same MLCs as the anti-DBA/2 T blasts; III: spleen cells from normal CBA/H mice.

† FCS: foetal calf serum; NMS: normal CBA/H serum; IS: serum from the CBA/H mice immunised with CBA/H-anti-DBA/2 T blasts. All sera 56 °C 20 min inactivated before use.

‡ ^{51}Cr -labelled T blasts of CBA-anti-DBA/2 (kad), CBA/H-anti-ACA (kaf) and CBA/H-ConA blast were used as target in a cytotoxic release assay run at 6 h at a 100:1 ratio effector/target cells.

Table 3 Serum from CBA mice immunised with CBA-anti-DBA/2 T blasts contain anti-CBA-anti-DBA/2 idiotype antibodies as detected in gel diffusion experiments

Absorbed with	Tested against:	Positive line
—	CBA normal serum	—
—	CBA-anti-DBA/2 serum	+
—	CBA-anti-DBA/2 MLC sup.*	+
—	Foetal calf serum	—
—	CBA-anti-DBA/2 MLC sup., A	—
—	B	—
—	C	+
—	D	+
—	E	—
—	CBA-anti-DBA/2 MLC sup.	—
—	abs with DBA/2 spleen	—
—	abs with CBA spleen	+
—	abs with ACA spleen	+
DBA/2 spleen cells	CBA-anti-DBA/2 MLC sup.	+
ACA spleen cells	CBA-anti-DBA/2 MLC sup.	+
CBA spleen cells	CBA-anti-DBA/2 MLC sup.	—

*MLC sup: supernatant from day 5 MLC of CBA-anti-DBA/2. Supernatant concentrated 100 times before testing in gel diffusion. Two different batches positive. Supernatant fractions A-E mean fractions after Sephadex G-200 fractionation. C and D: fractions in the size range of human serum albumin or slightly above, B: size range of IgG, A: size range of IgM.

antibodies as immunogen in the present system could be shown to lead to selective unresponsiveness to the relevant histocompatibility antigens⁵ we deem it most likely that the present unresponsiveness as measured in the MLC tests is induced through the same basic mechanism, namely that of autoanti-idiotypic immunity. Thus, the present experimental approach would seem to promise to provide a general tool to enrich *in vitro* for relevant antigen-binding, idiotype cells using the latter to induce productive auto-immune reactions leading to specific unresponsiveness to the relevant antigens. We are presently trying to study how this approach can be used in the induction of specific transplantation tolerance as well as in attempts to break existing states of delayed hypersensitivity.

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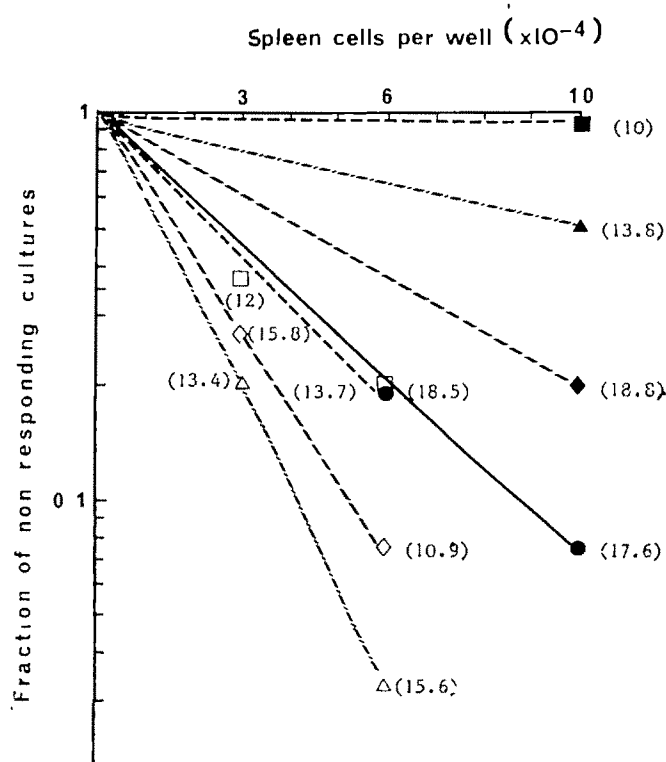
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Evidence for the inactivation of precursor B cells in high dose unresponsiveness

THE mechanisms of tolerance induction, a state of specific antigen induced unresponsiveness in lymphocytes, are still ill understood. The impetus to generate information on these mechanisms derives from the practical importance of understanding "self-tolerance". Both T (thymus-derived)

and B (bone marrow-derived) lymphocytes can be rendered specifically unresponsive to antigen. This paper examines the nature of the unresponsiveness induced in B cells by high doses of two types of antigen; firstly by a set of hapten conjugates known to be thymus-independent antigens (DNP-SIII, DNP-Levan, DNP-Dextran)¹ and secondly by a known thymus-dependent conjugate (TNP-BSA). It is now well authenticated that for efficient antibody production B cells need to proliferate after which members of the proliferated clone may differentiate into antibody producers. It is therefore important to establish whether unresponsiveness induced in B cells occurs by inactivation of the precursor cell or by an effect on the derived clone. We here demonstrate that *in vitro*, B cell unresponsiveness induced by both kinds of antigens (thymus-dependent and independent) results solely from the inactivation of the precursor hapten specific B cells, so that these cells cannot expand into

Fig. 1 The effect of 48 h of preculture of spleen cells with DNP-polysaccharide conjugates on the frequency of precursors responding to $0.1 \mu\text{g ml}^{-1}$ TNP-LPS. 1×10^7 spleen cells ml^{-1} from normal CBA mice were incubated with various conjugates in 50 mm tissue culture dishes (Falcon) in 5-ml volumes. After 48 h spleen cells were washed and spun through heat inactivated foetal calf serum. Varying numbers of such cells were supplemented with irradiated (1,200 rads from a ^{60}Co source) spleen cells and cultured for 3 d in micro-test plates in RPMI containing 5% foetal calf serum and additives². All cultures were challenged with $0.1 \mu\text{g ml}^{-1}$ TNP-LPS. Positive wells were then determined by PFC assay of the micro-cultures. In brief the culture tray was flooded with 10 ml Eagles HEPES medium, and washed. Each well was sampled and anti TNP-PFC were enumerated by a modification of the Jerne PFC assay using hapten-conjugated donkey erythrocytes (TNP-DRBC). Results are expressed as the fraction of non-responding wells for each cell input. Average clone sizes are displayed in the parentheses. Average clone sizes were estimated by first establishing the frequency of hapten reactive spleen cells using the Poisson formula⁴. From this value the total number of hapten-specific precursors (N) distributed into 30 wells can be estimated. Where Σ PFC is the total number of PFC found in 30 wells $\Sigma(\text{PFC}/N)$ is the average number of PFC per hapten-specific B cell. Average clone sizes are displayed in parentheses. ●, Nil; ■, $10 \mu\text{g ml}^{-1}$ DNP₂SIII; □, $0.1 \mu\text{g ml}^{-1}$ DNP₂SIII; ◆, $10 \mu\text{g ml}^{-1}$ DNP₂Levan; ◇, $0.1 \mu\text{g ml}^{-1}$ DNP₂Levan; ▲, $0.3 \mu\text{g ml}^{-1}$ DNP₂De; ○, $0.03 \mu\text{g ml}^{-1}$ DNP₂De.



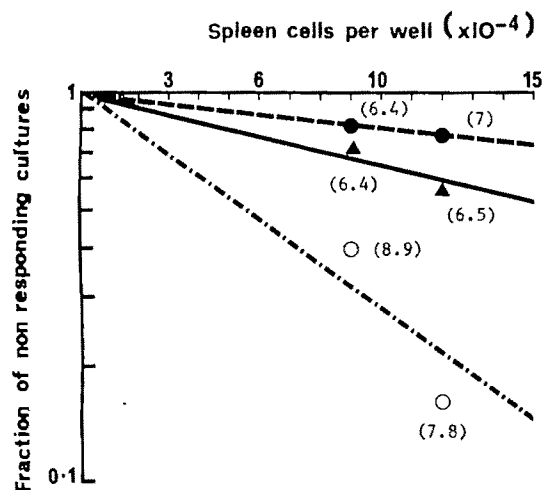


Fig. 2 The effect of 48 h of preincubation of spleen B cells with TNP₁₀BSA on the frequency of precursors responding to TNP-LPS and their secreting clone size. Spleen cells from adult thymectomised, lethally irradiated, bone marrow repopulated CBA mice were incubated for 48 h at 1×10^7 cells ml^{-1} as before with no TNP₁₀BSA (○), $50 \mu\text{g ml}^{-1}$ TNP₁₀BSA (▲), or $500 \mu\text{g ml}^{-1}$ TNP₁₀BSA (●). After this the cells were washed and redistributed as in Fig. 1. All wells were supplemented with irradiated AT×Bm cells to a final cell concentration of 1.5×10^4 per well. 30 wells were established for each treatment group. All microcultures were then challenged with $0.1 \mu\text{g ml}^{-1}$ TNP-LPS and anti-TNP responding cultures enumerated as before. Each point represents the mean of 30 microcultures. The conjugation ratio of TNP₁₀BSA is expressed as moles of hapten per mole of BSA.

normal antibody producing clones, rather than by any mechanism which may have affected the clone size such precursor cells could yield.

Spleen cells from CBA mice were passed through Sephadex G10 columns² to eliminate 'background' antibody producing cells (PFC). These cells were incubated with various concentrations of the hapten polysaccharide conjugates in bulk cultures ($1 \times 10^7 \text{ ml}^{-1}$ in 5-ml volumes) for 48 h and were then washed, established in microcultures in 10 μl volumes in microtest 3034 plates (Falcon)³, and re-challenged with optimal concentrations of Trinitrophenylated-lipopolysaccharide (TNP-LPS), another thymus-independent antigen. By titrating various numbers of such pretreated spleen cells into a filler population of irradiated cells it was possible to examine the frequency of the responding B cell using Poisson distribution analysis⁴. With the resultant knowledge of precursor frequency, and having calculated the total number of PFC a given number of spleen cells could produce, we were able to make estimates of average clone size.

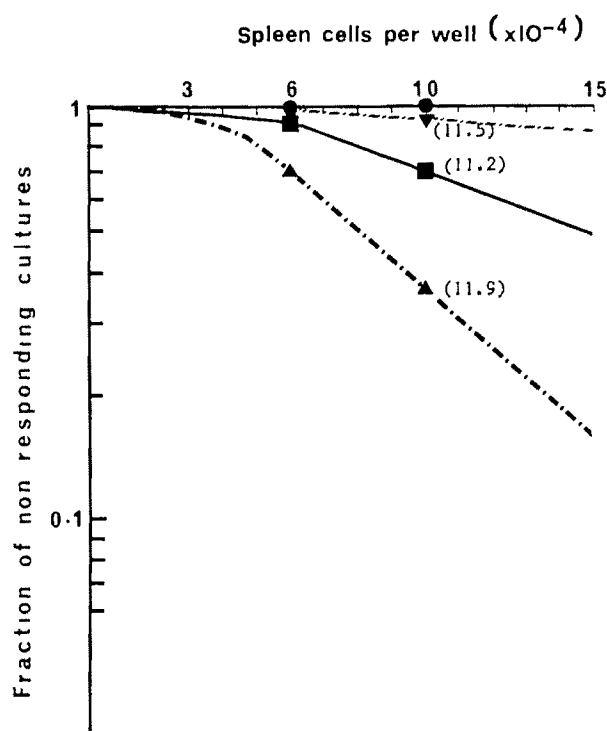
Figure 1 shows that cells precultured for 48 h in the presence of supraoptimal amounts of DNP-Dextran, DNP-Levan or DNP-SIII exhibited lower frequencies of cells reactive to TNP-LPS than controls. It should be pointed out that the pattern of responses observed with these three conjugates correlates well with their immunogenic and tolerogenic properties *in vivo*⁵ and in *in vitro* Marbrook cultures¹. Estimates of the average clone size of all pretreated groups responding to TNP-LPS showed that the number of PFC per anti TNP B cell was constant at approximately 10-19 PFC per precursor cell. It can be seen from Fig. 1 that the small variations in clone size estimates did not correlate with the tolerogenicity of the conjugates. This suggests that the reduced responses induced by high concentrations of T-independent antigens is primarily a consequence of inactivation of precursors rather than an influence on their subsequent clonal progeny.

An analogous situation was also observed after exposure of spleen cells from adult thymectomised irradiated and bone marrow repopulated CBA mice to high concentrations

of TNP₁₀BSA, a relatively oligovalent ligand. After a preincubation period of 48 h, free antigen was washed away, and frequencies and clone sizes of hapten-reactive B cells were determined on rechallenge with TNP-LPS. Preincubation of cells with $50 \mu\text{g ml}^{-1}$ TNP₁₀BSA produced more than a threefold reduction of hapten specific precursor cells reactive to TNP-LPS, whereas $500 \mu\text{g ml}^{-1}$ TNP₁₀BSA produced more than a sixfold reduction (Fig. 2). Estimates of the average clone size (PFC/precursor) are also shown in the inserts for each point in the figure, and were constant at approximately 6-8 PFC per B cell.

Similarly, if one examines the frequency of cells reactive to DNP₂₅Dextran (DE) in a normal spleen population using the microculture system, it is clear that at supraoptimal doses of antigen a diminished frequency of reactive cells is seen but clone sizes stay constant. Thus, in Fig. 3 is shown the data from an experiment where different concentrations of DNP-DE were added to groups of 30 microcultures, each group containing varying numbers of normal cells maintained at a constant cell concentration with the irradiated filler cells. After 4 d of culture both the number of wells giving a positive anti-DNP response and the total number of anti-DNP PFC generated for each group were determined. The frequency of reactive cells was approximately 1×10^{-4} with $0.03 \mu\text{g ml}^{-1}$ of DNP-DE but increasing concentrations resulted in lower frequencies of responding cultures, such that at $1 \mu\text{g ml}^{-1}$ no responding wells were detected (Fig. 3). Calculations of average clone size (that is

Fig. 3 The effect of various doses of DNP₂₅De (B1299) on the frequency of anti-TNP precursor B cells, and on the clone size such cells can generate. Sephadex G10 treated spleen cells from normal CBA mice were incubated in microcultures at various numbers in a final cell concentration of 10^7 ml^{-1} maintained by the addition of irradiated filler cells. Cultures were challenged with various concentrations of DNP-De. After 4 d of incubation wells were harvested and assessed for PFC as before. Results are expressed as the fraction of non-responding wells for each cell input. Each point represents the fraction of non-responding cultures from 30 wells sampled. Average clone sizes are displayed in parentheses in the figure. ▲, $0.03 \mu\text{g ml}^{-1}$ DNP-De; ■, $0.1 \mu\text{g ml}^{-1}$ DNP-De; ▼, $0.3 \mu\text{g ml}^{-1}$ DNP-De; ●, $1.0 \mu\text{g ml}^{-1}$ DNP-De. DNP-De was prepared as described previously and the conjugation ratio is expressed for molecular weight of 40,000.



PFC per B cell) were found to be relatively similar at all antigen concentrations where positive wells were detected (approximately 11 PFC/B cell). These values for clone sizes are again stated by each point in the figure. The data suggest that supraoptimal concentrations of this thymus-independent conjugate produced their inhibition of the anti-DNP response by inhibiting the precursor B cell without influencing the clones derived from those which escaped inhibition. Identical results have also been obtained with varying concentrations of DNP₂ levan.

These results obtained in the defined conditions of the microcultures lead us to conclude that the interaction of B cell surface receptors with high concentrations of multi-valent thymus-independent antigens results in a situation where the cell either enters into a division and differentiation 'programme' to generate a burst size constant for that antigen, or does not begin the cycle at all. It is clear that such inhibited cells are refractory to restimulation at least in the short term *in vitro* situation. B-cell unresponsiveness generated by the T-dependent ligand TNP-BSA is similar in this respect. It remains to be determined whether these refractory states of the precursor B cells are permanent or reversible.

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Lack of suppressive effect of α -foetoprotein on development of experimental allergic encephalomyelitis in rats

α -FOETOPROTEIN (AFP) is a major glycoprotein that occurs in substantial amounts in mammalian sera and body fluids during foetal and early neonatal life. Unusually high levels of AFP have been observed in the amniotic fluid in association with congenital abnormalities of the central nervous system (CNS)^{1,2}. Abnormal amounts have also been found in adults with hepatic and gastrointestinal malignancy^{3,4}. AFP has attracted attention as a potential immunoregulatory protein with suppressive activity for immune responses of mammalian lymphoid cells⁵⁻⁷, including autosensitisation. For example, normal rat serum prevents adult rat lymphoid cells from reacting immunologically to self-antigens *in vitro*⁸. In rats with experimental allergic encephalomyelitis (EAE), a prototypic autoimmune disease of the CNS⁹, a serum factor has been reported which causes suppression of proliferative responses of rat lymphoid cells exposed to mitogens¹⁰. Peak serum suppressive activity paralleled increasing α -globulin concentration and coincided with reversal of disease. We have reported¹¹ that development of EAE is impaired in suckling rats of an age when high levels of AFP would be expected. Using two rat model systems, we have dissociated

AFP production, assessed by serum concentration, from the occurrence of EAE. Our findings suggest that AFP does not have a major suppressive role in the development of this autoimmune disease.

We took advantage of two available model systems which utilise rats sensitised to nervous tissue-adjuvant emulsions. The first involves adult Buffalo strain rats bearing designated Morris hepatomas which produce diverse amounts of AFP¹², and the second involves the diminished occurrence of EAE in suckling Lewis rats 4-14 d old¹³.

To prepare the first model, adult female Buffalo rats, 8-12 weeks old, except in one experiment with 30-week-old animals, were implanted with cells from tumours that produced either high levels of AFP (Morris hepatoma 7777) or low levels of AFP (Morris hepatoma 5123)¹². Palpable tumours were usually evident within 2 or 3 weeks, at which time the tumour-bearing rats and uninoculated controls (of identical age and received in the same shipment of animals used for implantation of tumour cells) were sensitised to guinea pig spinal cord (GPSC) in complete Freund's adjuvant (CFA) as described before¹³.

To prepare the second model, suckling Lewis rats, an isohistogenic strain known to be not only highly susceptible to EAE¹⁴ but, from observations in our laboratory, far more susceptible than Buffalo rats, were similarly sensitised with GPSC-CFA at different times after birth¹⁵.

All rats were checked daily for clinical neurological signs—limp tail, ataxia and hindleg weakness or paralysis. Rats were killed and CNS tissue was collected and processed as follows. Formalin-fixed blocks of cerebrum, mesencephalon, cerebellum-pons and spinal cord (representing cervical, thoracic-lumbar and cauda-equina regions) were embedded in paraffin, sectioned at 5-7 μ m, stained with haematoxylin and eosin and examined microscopically for evidence of perivascular mononuclear cellular infiltrates characteristic of EAE. AFP levels in sera collected from normal or sensitised rats at appropriate times were measured by single radial diffusion in agar¹⁶.

In six experiments summarised in Table 1, Buffalo rats bearing Morris hepatomas had a lower incidence of both clinical signs and cellular infiltrates indicative of EAE compared with controls. This was true irrespective of which type of tumour had been inoculated. Neither the incidence nor the severity of EAE in individual rats could be related to the serum levels of AFP at the time of death. Moreover, there was no relationship in individual rats between incidence of EAE and the size of tumours (based on wet weight of masses excised at autopsy), or between tumour size and AFP serum levels at the time of death. From these data it was possible to conclude that AFP does not directly suppress the occurrence of EAE, and the diminished incidence of the disease is probably a result of the tumour *per se*.

As anticipated from somewhat higher AFP levels in 3-week-old compared with adult rats¹⁶, we found that neonatal Lewis rats had very high levels of serum AFP (Fig. 1), in

Table 1 Occurrence of EAE in Buffalo rats bearing Morris hepatomas

Type of tumour injected	Occurrence of EAE		AFP levels (μ g ml ⁻¹)*	
	Clinical signs	Cellular infiltrates	Range	Average
None	18/22	21/22	<20	<20
Morris 7777	4/11	6/11	1,320-4,130	2,194
Morris 5123	0/5	3/5	110-258	170

Rats were injected intracutaneously in six sites over the upper back and one site on the ventral neck (0.1 ml per site) with a 33% GPSC tissue homogenate in 0.25% phenol H₂O and emulsified with an equal volume of CFA (BCG 4 mg ml⁻¹).

*AFP levels at death, 19-24 d after sensitisation.

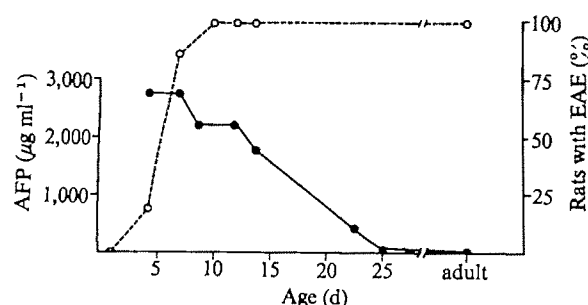


Fig. 1 AFP levels (●) and occurrence of EAE (○) in rats as a function of age. Suckling and adult Lewis rats were sensitised as described in Table 1, except that injection was into four sites over the upper back and one site on the ventral neck (0.05 ml per site) by the subcutaneous or intracutaneous routes.

the range of $2,750 \mu\text{g ml}^{-1}$ at 4 and 7 d of age and then gradually declining towards adult values after 25 d of age. Figure 1 also shows that 7-d-old or older suckling rats sensitised with GPSC-CFA at various ages were susceptible to EAE, although they had levels of AFP comparable with those of tumour-bearing rats (Table 1). There was no significant difference in the latent period for development of EAE (the time between sensitisation and the onset of clinical signs) in these suckling rats compared with adult rats. (A lengthening of the latent period in suckling rats might have represented a subtle manifestation of an AFP-mediated suppressive effect on development of disease, at least until AFP levels decline with increasing maturation (Fig. 1).) The fact that 7-d-old rats can develop EAE even with AFP levels comparable or identical to those of 4-d-old animals, which have a low incidence of EAE, is our most conclusive evidence that AFP does not explain the lack of disease in 4-d-old rats.

AFP concentrations considerably greater than normal occur in association with congenital malformations of the CNS^{1,2} and ataxia telangiectasia, an immunodeficiency disease involving the CNS¹⁷. We therefore investigated whether the injury to the brain and spinal cord which is characteristic of EAE is accompanied by increased concentrations of AFP in serum. Twenty-four adult 8–12-week-old Lewis and Buffalo rats were bled 12–24 d after being sensitised to GPSC-CFA, a time when inflammation occurs in the CNS in association with the appearance of clinical neurological signs of EAE. Levels of AFP were not appreciably different from those of adult rats before sensitisation (Fig. 1). Thus, injury to the CNS does not result in markedly increased AFP levels as occurs with ataxia telangiectasia and malformations of the CNS. Furthermore, AFP does not increase appreciably as part of the "acute phase reaction" accompanying inflammation irrespective of its nature, which is commonly observed with serum proteins, such as fibrinogen and complement.

The incidence of EAE in rats bearing Morris hepatomas was less than in simultaneously sensitised adult Buffalo rats bearing no tumours (Table 1). But disease was not suppressed in non-tumour-bearing suckling Lewis rats with comparable levels of AFP (Fig. 1). We suspect that the diminished incidence of disease associated with the Morris hepatoma reflects other tumour-related factors, such as diminished host inflammatory and/or immune responses which are known to be decreased in the presence of a critical burden of tumour cells¹⁸. AFP has been reported to have quite a variable suppressive effect on *in vitro* lymphocyte proliferation induced by different mitogens¹⁹. Other workers^{3–7} have suggested that AFP has an unequivocal suppressive effect *in vitro* on antibody production as well as on lymphoid cell proliferation induced by either allogeneic cells or mitogens. Studies *in vivo*, on the other hand, have demonstrated preferential suppression of IgA compared with IgG antibody responses to sheep red blood cells²⁰.

These various results suggest that the net suppressive effect of AFP in a given situation depends on the type of antigenic stimulus and the intensity of the resulting immune response. Vigorous antigenic stimulation with spinal cord and Freund's adjuvant as used to induce EAE, could conceivably bypass a potential suppressive effect of AFP in rats. In this regard it may be useful to determine the effect of AFP on other autoimmune diseases induced by different types of antigenic stimulation, such as thyroiditis, orchitis and adrenitis.

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Murine neuroblastoma cured *in vivo* by an antibody-dependent cellular cytotoxicity reaction

CYTOLYSIS of antibody-coated tumour cells mediated by reticuloendothelial (RE) or bone marrow cells is a phenomenon of unknown biological relevance^{1–4}. The specificity of the reaction lies in the antibody^{5,6}; probably most or all cells able to recognise the Fc fragment of the antibody molecule can mediate this reaction, including lymphocytes and monocytes^{6,7}, circulating cultured transformed lymphoblasts⁸, polymorphonuclear leukocytes⁹ and malignant reticulum cells¹⁰. In tumour systems the contribution of this reaction is difficult to distinguish *in vivo* from other cytolytic phenomena, including complement-dependent antibody-mediated cytotoxicity, T-cell cytotoxicity or cell killing exerted by "armed" macrophages^{11,12}, all of which may be active in various degrees in different experimental circumstances. But antibody-dependent cellular cytotoxicity (ADCC), as this phenomenon has been termed¹³, seems to be of potential therapeutic interest because both its magnitude and specificity depend on specific antibody levels, and antibody levels may be potentially more manipulable in man than are the cellular elements of a host anti-tumour response. In addition, there is preliminary evidence that

Table 1 *In vitro* ADCC against MNB cells

Sample	c.p.m.	±s.e.	⁵¹ Cr % Specific release
MNB cells, control	1140	38	0
MNB cells+RSC	1055	81	0
MNB cells, freeze-thawed	2019	55	100
MNB cells+AS 1:50+RSC	1528	34	44
MNB cells+AS 1:250+RSC	1570	44	49
MNB cells+AS 1:6250+RSC	1159	36	2
MNB cells+AS 1:31250+RSC	1088	26	0

Log phase MNB cells were collected by treatment with 0.25% Viokase (Grand Island Biological) for 2–5 min at room temperature. After aspiration and washing once in full growth medium, cells were resuspended in DMM to a final concentration of $2-4 \times 10^6$ ml⁻¹. They were then labelled with 300–400 μ Ci of sodium ⁵¹Cr-chromate (New England Nuclear Corp.) by incubating in tissue culture conditions in an incubator perfused with water-saturated 95% air: 5% CO₂ at 37 °C for 30 min with intermittent shaking every 10 min. The cells were then washed three times by centrifugation and resuspension in Hanks solution followed by final resuspension at 2×10^6 cells ml⁻¹ in DMM. Heat-inactivated (56 °C for 30 min) rabbit anti-MNB serum was then added to 1.0 ml of cells to give the final concentrations indicated. Each sample was then incubated at 37 °C for 30 min with shaking every 10 min. RSC were isolated by gentle mincing of a fresh spleen from an adult Wistar rat. RSC suspended in DMM (2×10^7 ml⁻¹) were then added (0.1 ml) to the antibody-coated ⁵¹Cr - labelled MNB cells to give a final ratio of 100:1 RSC:MNB cells. The mixture was then incubated for about 18 h at 37 °C in the tissue culture conditions described above. Each sample was then centrifuged for 10 min at 118g. A sample of the supernatant (0.5 ml) was then aspirated carefully from above the cell pellet and counted in a Beckman gamma scintillation counter. Duplicate MNB cell samples processed in an identical way without anti-MNB serum or RSC treatment were used to determine spontaneous ⁵¹Cr release. "Total" release was measured by freezing and thawing similar duplicate untreated samples three times. Non-immune serum was without effect.

%Specific release=

$$\frac{\text{c.p.m. test sample} - \text{c.p.m. control (MNB cells alone)}}{\text{c.p.m. freeze/thaw} - \text{c.p.m. control MNB alone}} \times 100$$

fixed tissue macrophages may be active in eliminating circulating metastatic cells by this mechanism¹⁴. It was therefore interesting to determine whether functional ADCC could be demonstrated *in vivo* independent of other cytolytic mechanisms since its demonstration has so far been limited to *in vitro* assays.

We used the C-1300 murine neuroblastoma (MNB) line carried in syngeneic A/J mice. Spindle-cell sarcoma I (SaI) was used as a control; SaI is also A-strain-derived and has a 100% transmission rate in syngeneic A/J mice. The MNB system is of special interest since C-1300 MNB cells remain capable of neuronal differentiation when passage *in vivo* many times^{15,17}. The disease closely resembles human neuroblastoma (HNB) both in this respect and in its response to chemotherapy¹⁸ and radiotherapy¹⁹. HNB is the most common solid tumour of early childhood with a peak incidence in the second year of life²⁰. It is characterised by a fulminant proliferation of immature sympathetic neuro-

blasts. Differentiated MNB cells have been shown to develop antigenic changes similar to those occurring during normal neuronal development^{21,22}. Most importantly, HNB patients and their mothers have circulating antibodies and cytotoxic lymphoid cells able to recognise HNB cells *in vitro*²³. Thus HNB patients potentially possess the active components for ADCC and augmentation of such a response might have therapeutic value. The experiments reported here indicate that ADCC with significant specificity can be demonstrated against MNB cells both *in vitro* and *in vivo* at levels which are therapeutically curative.

To obtain antisera against MNB cells adult female New Zealand rabbits were immunised by four injections of 10⁷ log phase MNB cells grown in Dulbecco's modified medium (DMM) *in vitro* as described before²⁴. Ten days after the fourth injection, the animals were bled and titred for anti-MNB activity. A single lot of antiserum was used for all the experiments. ADCC was first evaluated *in vitro* using a ⁵¹Cr-release method²⁵ outlined in Table 1. Neither antibody alone nor effector RE cells (rat spleen cells, RSC) were active alone while MNB cells coated with antibody and then exposed to RSC showed significant cell killing. The results indicated that ADCC was demonstrable in the system.

To determine whether ADCC could occur *in vivo*, MNB were first coated with antibody *in vitro* and cells were then injected subcutaneously into the right flank of syngeneic A/J mice. The ability of RSC cells to induce ADCC was then evaluated by a second injection of RSC in the general location of the original MNB injection. In each case separate needles, syringes and injection points were used. The animals were then watched for the time of appearance of palpable tumour (>1.0 mm) and subsequent survival. The pooled results (Table 2) of three separate experiments showed that neither *in vitro* antibody treatment nor RSC alone were effective. Antibody alone had essentially no effect ($P=0.62$), while RSC injected against non-antibody coated MNB cells delayed the onset of palpable tumour slightly with statistically significant lengthening of the median survival ($P=0.003$). The degree of protection was a function of the ratio of RSC to MNB cells injected. When antibody-coated MNB cells were studied, low ratios of RSC:MNB cells (10:1) led to a statistically significant additional increment in the delay of onset and median survival ($P<0.001$) while higher ratios (50:1) further extended the time of onset and survival of those animals which developed tumours. In addition, one out of every three animals treated at the 50:1 ratio of RSC:MNB cells failed to develop tumours. Because fewer than 10 MNB cells can induce lethal tumours in A/J mice²⁶ the results suggest that ADCC is highly efficient, having achieved about a 5 decade kill (which means that 1 in 10⁵ survive) in about one-third of the mice. This phenomenon was dependent on injection of the effector RSC in the general area of the original MNB

Table 2 *In vivo* ADCC against MNB cells

Sample	Median onset (d)	s.e.m. (d)	Median survival (d)	s.e.m. (d)	Cures
MNB alone	9.93	0.35	25.6	0.86	0/15
MNB+AS	10.53	0.42	26.2	0.84	0/15
MNB+RSC (10:1)	13.78	0.36	31.1	1.27	0/9
MNB+RSC (50:1)	14.3	0.40	32.4	1.25	0/10
MNB+AS+RSC (10:1)	16.13	0.78	33.5	1.44	0/15
MNB+AS+RSC (50:1)	21.54	1.77	37.6	1.71	5/15
MNB+AS+RSC (50:1) ectopic	11.8	0.73	28.4	2.29	0/5

Eight-week-old A/J mice were inoculated subcutaneously in the right flank with 10⁶ MNB cells in 0.1 ml which had been either pretreated (0.1 ml AS or dilution per 10⁶ cells for 60 min at 37 °C) with rabbit anti-MNB serum (AS) or not (MNB alone). The inoculating needle was withdrawn and in treated animals a second needle was inserted in the same general area and either 0.1 or 0.5 ml of RSC (10⁶ cells ml⁻¹) was injected to give the indicated ratios of RSC:MNB cells. In one series of experiments, the RSC cells were injected in the left flank (ectopic injection). The mice were then watched for the time of appearance of palpable tumour and for survival. Cures indicate those animals failing to develop tumours within 60 d. In the series in which "cures" were obtained only animals developing tumours were included when median survival was calculated. The paired *t* test was used to calculate statistical significance between each group and MNB controls.

Table 3 Specificity of *in vitro* ADCC against MNB cells

Sample	Median onset (d)	s.e.m. (d)	Median survival (d)	s.e.m. (d)
SaI alone	6.0	0.0	17.4	0.87
SaI+AS (ab)+RSC	6.2	0.20	19.0	1.58
MNB alone	11.8	0.73	31.2	1.32
MNB+AS (ab)+RSC	22.2	2.03	48.8	2.92

A/J mice were injected with either uncoated SaI or MNB cells as described in Table 2. Another set of mice received similar inoculations after coating with anti-MNB serum which had previously been repeatedly absorbed with SaI cells (10^7 per ml of anti-MNB serum) until complement-dependent antibody mediated cytotoxicity was not demonstrable by Trypan blue exclusion. The animals receiving AS-coated cells subsequently received an inoculation of RSC (50:1) at nearby site, as described in Table 2.

injection. Ectopic injection into the left flank of recipient mice was essentially without effect (Table 2).

To check the specificity of the reaction, the anti-MNB serum was absorbed exhaustively with SaI cells as described in Table 3. Absorbed antisera had no effect on either the time of tumour appearance or the time of death of mice receiving SaI cells and high levels of RSC. The antisera, however, were still effective in delaying the time of appearance of MNB tumours ($P < 0.01$) and the time of death of the animals ($P < 0.001$).

These experiments suggest that the MNB system is interesting in the overall approach to HNB treatment and also as a general approach to the study of *in vivo* ADCC phenomenon as a potential means of cancer therapy. The data indicate clearly that ADCC does not occur only *in vitro* but also *in vivo*. But to be effective a relatively high ratio of effector cells is required and these must be relatively near the target cells. Ectopic exposures of even favourable effector cell ratios were ineffective (Table 2). But with appropriate antisera and physical circumstances, the effect is specific and very striking. In optimum conditions the extent of tumour cell killing greatly exceeds that attainable by aggressive chemotherapy¹⁸ and has no detectable deleterious effect on the "cured" mice. It is very interesting that a small amount of xenogeneic antiserum that is itself ineffective (that is not rendered cytotoxic by *in vivo* exposure to mouse complement) can be curative in the ADCC system. *In vitro* activation of armed macrophages was reported by Van Loveren and Den Otter. Although the nature of the activating substance was not identified it also proved therapeutically effective in rendering normal macrophages tumoricidal²⁷.

We suggest that *in vitro/in vivo* modes for evaluating ADCC may be useful in the evaluation of multidisciplinary cancer therapy. For example, it is well established that MNB is highly resistant to *in vivo* chemotherapy, as is its human counterpart¹⁴. Multimodality treatment regimes can therefore be evaluated by this type of model. We have shown that liver parenchymal RE elements (presumably Kupffer cells) can effect moderate levels of tumour cell killing by means similar to or identical with ADCC¹⁴. The results shown here indicate that tumour cell killing *in vivo* by ADCC mechanisms can be active in tissues but is subject to significant anatomical limitations. We propose that human tumours with favourable anatomical sites (such as ovarian carcinoma) may benefit from this approach. The *in vitro/in vivo* model such as exemplified here should facilitate evaluation of specific tissue limitations and perhaps the study of means of stimulating RE cell migration in abrogating such limitations. Unlike T-cell-mediated immunity, the specificity of ADCC is antibody-mediated. Species barriers may therefore be less of a problem during passive immunotherapy because xenogeneic antiserum is clearly effective. Moreover, passive transfer of immunity ("arming") by antigen-antibody complexes is a characteristic of this type of immunity^{11,28}. These features suggest that the ADCC reaction may prove manipulable in human

tumour immunotherapy and warrants further exploration in this context.

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Serological reactions against glycolipid-sensitized liposomes in multiple sclerosis

MULTIPLE SCLEROSIS (MS) is a neurological disease of unknown aetiology which involves the destruction of central nervous system myelin¹. Although recent results have implicated a possible viral agent^{2,3}, the search continues to identify an antigen which might provoke a postulated autoimmune reaction⁴. Such a role has been suggested not only for proteins, but recently also for myelin glycolipids, particularly galactocerebroside^{5,6}. However, to demonstrate immunological reactions against lipid antigens presents special problems: solubility, accessibility of reactive groups,

the anticomplementarity of many lipids and the requirement for auxiliary lipids, lecithin and cholesterol⁷. We have used a model membrane system which obviates these difficulties to survey sera from MS patients for reactivity against crude extracts of white matter lipids as well as various glycolipids. This test demonstrated antibodies reacting against cerebroside, gangliosides and white matter lipids in some of the MS sera tested, but reactions against digalactosyl diglyceride (an α -galactoside) were more frequent, and were found more often in MS patients than in normal controls, or those with other neurological diseases.

The assay employing liposomes (vesicles consisting of concentric lipid bilayers entrapping between them an aqueous compartment) is based on the fact that these model membranes can be lysed, in the presence of complement, by appropriate antisera against lipid haptens in their membranes⁸. The system seemed promising as a tool in the search for anti-lipid antibodies in MS sera, because it has been used to investigate reactions against several galactolipids in immunised rabbits⁹ and to identify antibodies to lipids of *Schistosoma* parasites¹⁰.

Multilamellar, unsonicated liposomes containing distearoyl lecithin, cholesterol and dicetyl phosphate in a molar ratio of 2:1.5:0.2, sensitised as described⁸, and incorporating umbelliferyl phosphate as a marker¹¹, were prepared. Lysis was measured by the inclusion, in the external medium, of alkaline phosphatase which converts released marker to fluorescent umbelliferone. The method of Six *et al.*¹¹ was scaled down ten-fold to obtain greater sensitivity (the preparation of unilamellar liposomes was not found necessary). Results were expressed as percentage of total marker released. Total entrapped marker was determined each day by lysing 1 μ l of liposomes in acetone. Controls without serum, or containing heated complement, were analysed in parallel, as well as unsensitised liposomes. (Any lysis obtained with these was subtracted.) Liposomes made with dipalmitoyl lecithin gave similar results to those containing distearoyl lecithin.

Specific antisera to the lipids used were raised in rabbits⁹ to determine the sensitivity of each batch of liposomes. Cross-reactivity experiments with these were in accord with published findings⁹; anticerebroside sera cross reacted to some extent with liposomes containing digalactosyl diglyceride (DGD), but anti-DGD sera did not lyse liposomes containing cerebroside or gangliosides. Sera from rabbits immunised with normal human white matter (NHWM)

lysed liposomes sensitised with galactocerebroside but not glucocerebroside, confirming results obtained by other methods¹².

Human sera were obtained through the services of the University of California at Los Angeles MS clinic. They were heated (56 °C, 30 min), divided into small aliquots and stored at -20 °C.

Initial experiments with MS sera showed that: (1) sera which lysed NHWM liposomes also reacted with cerebroside and gangliosides; (2) galactocerebroside liposomes were not lysed preferentially (only eight out of 23 patients tested gave positive reactions); (3) liposomes sensitised with digalactosyl diglyceride (DGD), however, were lysed by some sera which did not react with other lipids. Subsequent experiments were therefore conducted only with DGD and NHWM liposomes.

Positive reactions against these were found both in MS patients, normal controls, and patients with amyotrophic lateral sclerosis (ALS), myasthenia gravis (MG) or unspecified neurological disease. However, such reactions were more frequent among MS patients and were quantitatively greater (Table 1). The percentage of lysis in a liposome assay is a quantitative measure analogous to antibody titre⁴, as long as the maximal value obtainable with a specific batch of liposomes (usually about 50%) is not exceeded.

Patients whose sera were reactive did not fall into any specific group with regard to disease classification (progressive, relapsing, or relapsing and progressive), and the degree of lysis obtained was similar when blood was drawn on different dates. Three positive patients were sampled six times and always reacted similarly; negative patients remained so on several subsequent samplings.

The experiments reported above do not support the hypothesis that galactocerebroside or other known galactolipids of human white matter may act as antigens provoking an autoimmune attack on myelin in MS. DGD would appear to be a possible exception. Galactosyl diglycerides, once thought to be exclusively plant lipids, occur in brain, but in no other animal tissues, and are specifically localised in myelin^{13,14}. DGD, in contrast to monogalactosyl diglyceride (MGD), has not been identified with certainty in brain, but it is synthesised by brain microsomes. Both the β -galactose transferase which synthesises MGD and the α -galactose transferase which transforms it into DGD are brain specific, especially abundant in oligodendrocytes, and their activities parallel the process of myelination during development¹⁴.

Table 1 Frequency of reactions against sensitised liposomes

	Liposomes sensitised with Digalactosyl diglyceride		Normal human white matter lipids	
	Positives/individuals tested	Mean % lysis* \pm s.d. (positives only)	Positives/individuals tested	Mean % lysis* \pm s.d. (positives only)
MS	60/99	19.6 \pm 19	37/71	8.2 \pm 6
All controls	33/82	10.1 \pm 8†	21/77	5.8 \pm 4‡
Normal	18/42		4/33	
Amyotrophic lateral sclerosis	9/26		12/26	
Myasthenia gravis	4/8		3/10	
Unspecified neurological disease	2/6		2/8	

Liposomes were made from distearoyl lecithin, cholesterol and dicetyl phosphate in a molar ratio of 2:1.5:0.2 and contained 10 mM umbelliferyl phosphate in the aqueous phase¹¹. They were sensitised with 150 mg of digalactosyl diglyceride (Applied Science) per μ mol of lecithin, or with a chloroform-methanol (2:1) extract of normal human white matter equivalent to 0.6 mg fresh weight. (In the latter case, the ratio of dicetyl phosphate was increased to 0.4.) 1 μ l of the liposome preparation was added to 100 μ l of a complement buffer (20 mM Tris, pH 8.0, with 150 mM NaCl, 0.5 mM MgCl₂ and 0.15 mM CaCl₂) containing 50 μ l per ml guinea pig complement (Grand Island Biological) and 10 μ l per ml alkaline phosphatase. After addition of 5 μ l serum, they were incubated (30 min, 37 °C); 1 ml of the buffer was added before fluorescence measurement.

* %Lysis = $\frac{\text{fluorescence of marker released} \times 100}{\text{fluorescence from total marker entrapped}}$
(Lysis of unsensitised liposomes was subtracted.)

† Significant at $P < 0.005$.

‡ Probably not significant ($P < 0.1$).

Table 2 Absorption of rabbit antisera

Rabbits immunised with:	Normal human white matter	White matter from MS patient
		% Lysis
Unabsorbed	47.5	48.5
Absorbed with normal white matter	15.5	3.2
MS white matter	14.0	4.2

Liposomes were sensitised with an extract of normal white matter. Rabbits were immunised by injecting 0.5 ml of an emulsion made from 15 mg of lyophilised white matter, 0.25 ml complete Freund's adjuvant and 0.25 ml normal saline (foot-pads and back) and bled 3 weeks later. Sera were absorbed overnight with 30 mg of lyophilised white matter at 4 °C.

If DGD occurs in brain, it is the only known glycolipid which is an α -galactoside¹⁴.

Antibodies of different specificity may be involved in the reaction against DGD and β -galactolipids: (1) rabbit anti-DGD sera do not cross react with cerebroside or gangliosides⁶; (2) many sera which reacted with DGD did not lyse other liposomes; (3) when three reactive sera were absorbed overnight with suspensions of the lipids, only DGD abolished the reaction, while MGD, cerebroside, gangliosides and NHWM were inactive.

Lysis of DGD liposomes may reflect cross reactions with some other glycolipid. An attempt to detect an antigenic difference between the lipids of MS white matter and normal white matter, however, yielded negative results (Table 2).

Circulating antibodies of white matter lipids are likely to be a non-specific phenomenon resulting from the destruction of myelin; they were found in other neurological diseases as well as in normal individuals. Moreover, elevated levels of antibodies to measles and other antigens have been reported in MS (refs 15 and 16). The differences found between MS patients and controls are not sufficient to constitute evidence of a specific antigenic role of DGD in this disease. On the other hand, they would indicate the need to study the occurrence, concentration, localisation and function of this lipid in the nervous system. Our results also illustrate the usefulness of the liposome assay for the detection of antibodies against cell surface glycolipids, which is of interest in cancer immunology.

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Effect of botulinum toxin on trophic regulation of acetylcholine receptors

THE distribution of acetylcholine (ACh) receptors in mammalian skeletal muscle is regulated to a large extent by motor nerves¹. In innervated muscles, ACh receptors are localised almost exclusively at neuromuscular junctions, but after denervation there is a great increase of extrajunctional receptor density²⁻⁵. The trophic mechanisms by which motor nerves normally control extrajunctional ACh receptor density are not well understood. Although ACh transmission and the muscle usage it produces have been shown to play an important role⁶⁻¹¹, it has also been suggested that unrelated factors (for example, substances carried by axonal transport) exert some trophic regulatory influence on extrajunctional ACh receptors¹²⁻¹⁴. Since more than one factor may participate in this regulation, it is important to evaluate the relative contributions of each in quantitative terms. We have therefore made a quantitative comparison of the effects of botulinum toxin and surgical denervation on ACh receptors. Botulinum toxin blocks quantal release of ACh at nerve terminals¹⁵ in a highly specific manner that is thought to involve the vesicle release mechanism¹⁶. An ¹²⁵I- α -bungarotoxin binding technique was used for quantitative determination of ACh receptor density¹⁷. The results indicate that blockade of ACh transmission by botulinum toxin produces a partial denervation-like increase in extrajunctional ACh receptors, similar to, but somewhat greater than that seen after disuse alone¹⁷.

Injections of botulinum toxin, denervation or control procedures were carried out in 148 female Sprague-Dawley rats (190-215 g) under chloral hydrate anaesthesia (400 mg kg⁻¹). Botulinum toxin (supplied by Dr E. Schantz), freshly diluted in Ringer solution, was injected into the right extensor digitorum longus (EDL) or soleus muscle through a fine No. 30 needle on day 0. Each injection of 30 μ l contained 1.2×10^{-8} g of crystalline type A botulinum toxin¹⁸. For comparison, surgical denervation was produced by avulsing the sciatic nerve approximately 1 cm from the EDL and soleus muscles. Control animals received injections of boiled (inactivated) botulinum toxin. Two further groups of 10 rats were both denervated and injected with botulinum toxin.

At the time of the final experiment (0, 4 and 7 d) the rats were anaesthetised, and neuromuscular transmission was tested in all botulinum-treated muscles. The muscle was removed with the nerve attached, and placed in an oxygenated bath containing Trowell's T8 medium. The nerve was stimulated electrically, using stimulus parameters of 50 V, 100 Hz and 2 ms, and the muscle response was observed under the stereomicroscope. Only muscles that were completely paralysed were used for receptor determinations. Intracellular microelectrode studies were carried out in 17 of these nerve-muscle preparations at various times after botulinum injection. None of the paralysed muscles showed muscle fibre action potentials in response to nerve stimulation, although very small subthreshold endplate potentials (e.p.p.s) (mean amplitude = 0.41 ± 0.07 mV, maximum = 2.5 mV) were recorded intermittently. Neuromuscular blockade was well established within 3-6 h of treatment, and lasted throughout the experimental period.

Miniature endplate potentials (m.e.p.p.s) were recorded

in 26 botulinum-treated muscles. The m.e.p.p. frequency was reduced to 5–10% of normal within 3 h in the soleus and 6 h in the EDL, and rose only slightly by the end of the experimental period (Table 1).

We studied the effects of botulinum treatment on fast axonal transport, since this mechanism has been thought to play a role in neurotrophic regulation^{8,13,18}. Transport of protein and glycoprotein was measured in the sciatic nerves of botulinum-treated rats after injection of ³H-leucine or ³H-fucose into the lumbar ventral horns. In eight rats treated by injection of botulinum toxin 48–144 h previously into all the muscles supplied by the sciatic nerve, the rate of fast transport was normal (383.9±25.4 mm per 24 h)¹⁰ and equal to that of the opposite control side. Delivery of ³H-fucose-labelled glycoproteins to the motor nerve endings was also similar in control and botulinum-treated muscles of three rats, as shown by autoradiography of hindfoot lumbrical muscles 24 h after labelling of the ventral horns²¹. Thus the effects of botulinum toxin in these experiments cannot be attributed to interference with either fast axonal transport or delivery of materials to neuromuscular junctions.

Extrajunctional ACh receptor density was measured by a method which utilised saturation binding of ¹²⁵I- α -bungarotoxin^{17,22}. The specific and irreversible binding of this snake venom fraction to ACh receptors allows quantitative measurement of receptor density²³.

The results (Table 2) show that botulinum toxin produced an elevation in extrajunctional ACh receptor density in both the EDL and soleus muscles. The receptor density increased throughout the time studied, being greatest at 7d. This finding is consistent with the earlier observations on extrajunctional sensitivity to iontophoretically applied ACh (ref. 7). The increase in ACh receptors, however, was significantly smaller at the times tested than that produced by denervation (Table 2). Treatment of muscles with denervation plus botulinum toxin resulted in levels of extrajunctional ACh receptors like those of denervation alone. This indicates that the toxin did not inhibit ACh receptors. Furthermore, control injections of inactivated (boiled) toxin did not result in an increase of ACh receptor density, indicating that the experimental procedure itself could not account for the effect of botulinum toxin.

The simplest interpretation of our findings is that the known action of botulinum toxin in blocking neural ACh release is responsible in some way for the observed increase of extrajunctional ACh receptors. Botulinum toxin effectively blocked all suprathreshold ACh release throughout the experimental period, thereby eliminating nerve-induced usage of muscle. In addition, it greatly reduced subthreshold quantal ACh release (m.e.p.p.s and e.p.p.s), as described above. Recent evidence from our laboratory indicates that disuse alone, brought about by local block of nerve conduction, results in a similar but smaller increase of extrajunctional receptors¹⁷. This suggests that the main but

Table 2 Effects of botulinum on extrajunctional ACh receptor density

Treatment		Soleus muscle	
		4 d	7 d
Untreated	22±2 (30)		
Denervation		201±15 (9)*	413±43 (10)*
Botulinum		138±20 (8)†*	278±34 (9)†*
Boiled botulinum		30±10 (3)‡	17±5 (3)‡
Botulinum and denervation			420±35 (5)*§
Extensor digitorum longus			
Untreated	10±1 (36)		
Denervation		219±44 (5)*	420±24 (7)*
Botulinum		42±11 (6)*†	235±48 (6)*†
Boiled botulinum		6±1 (4)‡	12±2 (3)‡
Botulinum and denervation			435±38 (5)*§

Results are expressed as ACh receptors per μm^2 (mean±s.e.m.). Figures in parentheses indicate numbers of muscles.

*Greater than normal $P<0.001$.

†Less than denervation $P<0.02$.

‡Less than denervation $P<0.005$.

§Not different from denervation $P>0.1$.

not the only denervation-like effect of botulinum toxin on ACh receptors is due to the disuse it produces.

Because the effect of botulinum toxin on extrajunctional ACh receptors was less than that of denervation, some trophic influence must remain unblocked in the botulinum-treated nerves. Many possible factors have been suggested, including substances carried by axonal transport^{8,13,18}, persistent quantal and non-quantal ACh release, and nerve-muscle membrane interaction^{6,14}. Further studies are needed to clarify the role, if any, of each of these factors.

The effect of cholinergic blockade on ACh receptors does not necessarily parallel its effects on other trophically regulated muscle properties. For example, botulinum treatment completely reproduces the effect of denervation with respect to one such property, isometric contraction of muscle²⁴; but seems to have no effect on another, neuromuscular junction formation in the developing embryo²⁵. Thus, the factors involved in trophic regulation of each muscle property must be evaluated individually.

We conclude that neurotrophic regulation of extrajunctional ACh receptors involves cholinergic transmission, including muscle usage triggered by it. But some additional factor must also play a role.

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Table 1 Effects of botulinum on m.e.p.p. frequency and amplitude

Time	Soleus muscle	
	Frequency*	Amplitude†
0	0.97±0.14	0.38±0.02
3 h	0.16±0.04	0.38±0.05
6–72 h	0.11±0.02	0.28±0.02
7 d	0.16±0.03	0.30±0.04
Time	Extensor digitorum longus	
	Frequency*	Amplitude†
0	2.11±0.37	0.42±0.03
3 h	1.12±0.17	0.33±0.03
6–72 h	0.11±0.02	0.39±0.07
7 d	0.24±0.01	0.43±0.03

*Mean m.e.p.p.s±s.e.m. per s.

†Mean m.e.p.p. amplitude±s.e.m. corrected to 75 mV RMP.

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Is drug inhibition of dopamine uptake a misinterpretation of *in vitro* experiments?

COYLE and Snyder¹ reported that various antiparkinson drugs inhibit the uptake of labelled dopamine (DA) into striatal synaptosomes. They suggested that these drugs may act in part by a potentiation of dopaminergic neurotransmission caused by inhibition of DA uptake. Measurement of drug effects on the uptake of labelled DA into slices or synaptosomes from the corpus striatum has become a widely used test for the evaluation of drugs affecting central dopaminergic functions. Some observations made in our laboratory, however, suggest that the apparent reduction of ³H-DA uptake caused by a variety of drugs in *in vitro* experiments may, in fact, be the result of a DA-depleting action. Benztropine and nomifensine are said to be potent DA-uptake inhibitors²; however, in our experience, benztropine (30 mg kg⁻¹ subcutaneously) does not inhibit, and nomifensine (100 mg kg⁻¹ orally) only slightly inhibits, the uptake of ³H-DA into striatal synaptosomes prepared from rats pretreated 1 h before killing. In contrast, pretreatment with tricyclic antidepressants does inhibit NA and 5-hydroxytryptamine (5-HT) uptake into midbrain synaptosomes³. Using the method of Farnebo⁴, we studied the effects of benztropine, haloperidol, chlorpromazine, *d*-amphetamine, piroheptine⁵ and cocaine on the release of tritium from field-stimulated, ³H-DA-prelabelled striatal slices. We observed that, at concentrations between 10⁻⁶ M and 10⁻³ M, these drugs increase the spontaneous release of tritium soon after their addition to the superfusion medium.

In the experiments reported here, ³H-DA accumulation *in vitro* was measured in crude synaptosome preparations as described by Coyle and Snyder¹. The corpus striatum of male albino rats of a Sprague-Dawley strain (180-200 g) was homogenised in 0.3 M sucrose solution (1:10 w/v) as described by Whittaker⁶. The homogenate was centrifuged at 1,000 g for 10 min and the supernatant fraction was used. The following components were mixed in this

sequence at 0 °C: 5 ml Krebs-Ringer bicarbonate solution saturated with 95% oxygen, 5% CO₂ (ref. 7), containing per 100 mg ascorbic acid, 25 mg EDTA and 12.5 mg pargyline; drug to be tested, dissolved in 0.5 ml H₂O; 0.5 ml of the synaptosome suspension; and finally ³H-DA (New England Nuclear, specific activity 3.8 Ci mmol⁻¹), dissolved in 0.5 ml H₂O. The final concentration of ³H-DA was 6 × 10⁻⁸ M and the exogenous amine amounted to ~4% of the total endogenous DA content of the synaptosomes in the incubation. After incubation for 10 min at 37 °C, the synaptosomes were cooled to 0 °C, centrifuged for 15 min at 8,000g, washed with Krebs-Ringer solution, and recentrifuged. The pellet was homogenised in 10% TCA, and the homogenate centrifuged at 12,000g. DA was adsorbed on to alumina at pH 8.6 and eluted with 0.25 N HCl. An aliquot of the extracted catecholamines was counted with 10 ml Insta-Gel, and a second aliquot taken for the automated fluorimetric determination of DA according to the method of Waldmeier *et al.*⁸.

The results of these experiments are summarised in Fig. 1. The following drugs were tested at various concentrations: benztropine, nomifensine, piroheptine, haloperidol, chlorpromazine, diphenylhydramine, clomipramine, cocaine and *d*-amphetamine. In Fig. 1, ³H-DA uptake is plotted against DA content and it can be seen that there was a highly positive correlation between the apparent inhibition of ³H-DA uptake and DA depletion (*r*=0.986).

To see whether the observed phenomenon was restricted to DA uptake into striatal synaptosomes, we compared the effects of several psychotropic drugs on NA content and ³H-NA uptake, using midbrain synaptosomes from rat brain. The method adopted was similar to that used in the experiments with DA and striatal synaptosomes. The added ³H-NA (New England Nuclear, specific activity 4.8 Ci mmol⁻¹, final concentration 6 × 10⁻⁸ M) amounted to about 25% of the total endogenous NA content. The following drugs were tested: desipramine, clomipramine, nomifensine, chlorpromazine, *d*-amphetamine and cocaine and the results are shown in Fig. 2 in the same manner as those of Fig. 1. No depletion of NA was observed with most drugs in concentrations that markedly inhibited NA uptake. Some depletion of NA occurred with very high concentrations (10⁻³ M) of desipramine (28%) and with 10⁻⁴ M *d*-amphetamine (19%). The depleting action of *d*-amphetamine can be explained by the well known NA-releasing effect of this drug, observed *in vivo* in experiments performed with the push-pull cannula technique^{9,10}.

Fig. 1 Comparable effects of psychotropic drugs on ³H-DA uptake and endogenous DA content in rat striatal synaptosomes after 10 min incubation at 37 °C. Estimations of uptake and content were carried out in each case in the same synaptosome suspension. Each point represents the mean of 2-4 estimations. Ordinate: ³H-DA uptake as a percentage of the corresponding control values (80,000-100,000 d.p.m. per sample, tissue: medium ratio for control ³H-DA uptake, 80-100:1). The value obtained in the presence of 10⁻³ M benztropine (37 °C) was taken as nonspecific background uptake (about 6% of the uptake of the control). Abscissa: Endogenous content of DA as a percentage of the corresponding control values. The absolute values of the same controls as used for determination of ³H-DA uptake ranged between 130 and 170 ng DA per sample.

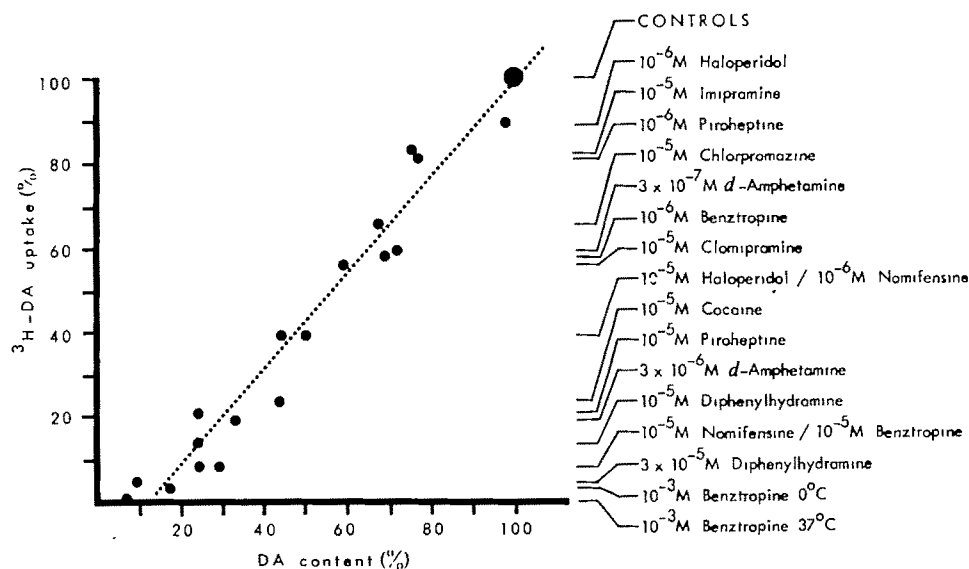
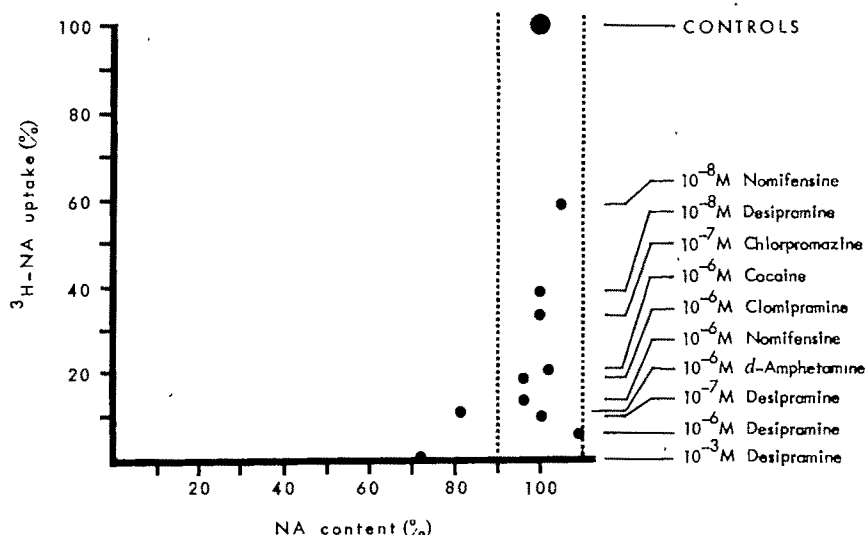


Fig. 2 Comparable effects of psychotropic drugs on ^3H -NA uptake and endogenous NA content in synaptosomes from rat mid-brain (including diencephalon) after 30 min incubation at 37 °C. Estimations of uptake and content were carried out in the same synaptosome suspension. Each point represents the mean of two estimations. Ordinate: ^3H -NA uptake as a percentage of the corresponding control values (21,000 d.p.m. per sample, tissue: medium ratio for control ^3H -NA uptake, 17:1). The values obtained in the presence of 10^{-8} M desipramine were taken as unspecific background uptake (about 15% of the uptake of the control). Abscissa: Endogenous content of NA as a percentage of the corresponding control values. The absolute value of the same controls as used for determination of ^3H -NA uptake was 22.5 ng NA per sample.



This effect was also observed *in vitro* in our laboratory with 10^{-8} M *d*-amphetamine in experiments in which superfused ^3H -NA-prelabelled cortical slices were used.

All the drugs commonly assumed to be DA-uptake inhibitors that we have tested so far have proved to be effective in releasing endogenous DA from striatal synaptosomes. Our data support the suggestion of Orlansky *et al.*¹¹ and Heikkilä *et al.*¹² that the so-called DA-uptake inhibitors are in fact DA-releasing agents, and that the apparent inhibition of DA uptake can be explained by drug-induced depletion of DA stores *in vitro*. They also bear out these earlier conclusions that a drug can only be designated as a DA-uptake inhibitor if a depleting effect has been excluded. On the other hand, it seems that the uptake of ^3H -NA into synaptosomes or slices from parts of the brain other than the striatum is a valid model for measuring NA-uptake inhibition.

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Release of substance P from isolated nerve endings

DISCOVERED in 1931 by von Euler and Gaddum¹ and purified to homogeneity in 1970 by Chang and Leeman², substance P is an undecapeptide with the sequence $\text{H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH}_2$ (ref. 3). Its function is still unknown but the selective distribution of substance P in the nervous system^{4–6}, its localisation in

nerve endings (refs 7–9 and our own unpublished results) and its neuropharmacological actions^{10–14} strongly suggest that substance P may participate, directly or indirectly, in the transmission process of certain neurones. However, if substance P is a neurotransmitter, it must also be released from nerve terminals upon the appropriate stimulation. In this report we present evidence showing that substance P is released from isolated nerve endings by depolarisation with high K^+ and that this release is calcium dependent.

These experiments were performed on synaptosomes, a preparation which has been useful in the study of the release of other putative neurotransmitters in the central nervous system¹⁵. Synaptosomes or crude synaptosomal (P_2) pellets were prepared according to Scheme II of Cotman¹⁶, starting with hypothalamic and ventral mesencephalons of adult male rats (Charles River Breeding Co., Wilmington, Massachusetts). The tissue was placed on a filter and superfused with a Ca^{2+} -free physiological salt solution. After the initial washout, the synaptosomal tissue was periodically exposed to brief pulses of test solutions. The superfusates were collected and the substance P content determined by radioimmunoassay¹⁷. In our initial studies we found no difference between synaptosomal fractions and crude synaptosomal (P_2) pellets with regard to their pattern of substance P release. Furthermore, none of the remaining fractions of the sucrose-Ficoll gradient used to prepare synaptosomes contained appreciable amounts of substance P. All data presented here were obtained with crude synaptosomal (P_2) pellets.

Since synaptosomes have a K^+ diffusion potential, depolarisation can be achieved by superfusing them with a high K^+ medium¹⁸. Only in the presence of Ca^{2+} does exposure to 60 mM K^+ induce a significant release of substance P (Fig. 1). This is analogous to the conditions that have been described for the release of established and putative transmitters as well as for several hormones¹⁹. Within our time resolution the evoked release of substance P occurs immediately and subsides after the removal of the stimulus (Fig. 2). It is interesting to note that a second stimulating pulse, administered after a brief interval, invariably released less substance P than the first (Fig. 2) and a third less than the second (not shown). This reduction in release is not due to a K^+ - or time-dependent deterioration of the tissue since prior exposure to high K^+ in the absence of Ca^{2+} had no effect on the evoked release and since a stimulating pulse was as effective at the end of an experimental run as at the beginning (see legend to Fig. 1). The decreased release may indicate a limited size of the substance P pool available for release or the gradual

inactivation of the Ca^{2+} dependent depolarisation-secretion coupling process giving access to that pool.

Microsomes, normally remaining in the supernatant when a crude synaptosomal pellet is obtained²⁰ sometimes contaminate the P_2 pellets. Because an appreciable amount of substance P is associated with this fraction (ref. 9 and our own unpublished results), we tested it for release of substance P. The small amount retained on the filters could not be released by high K^+ in the absence of Ca^{2+} . We

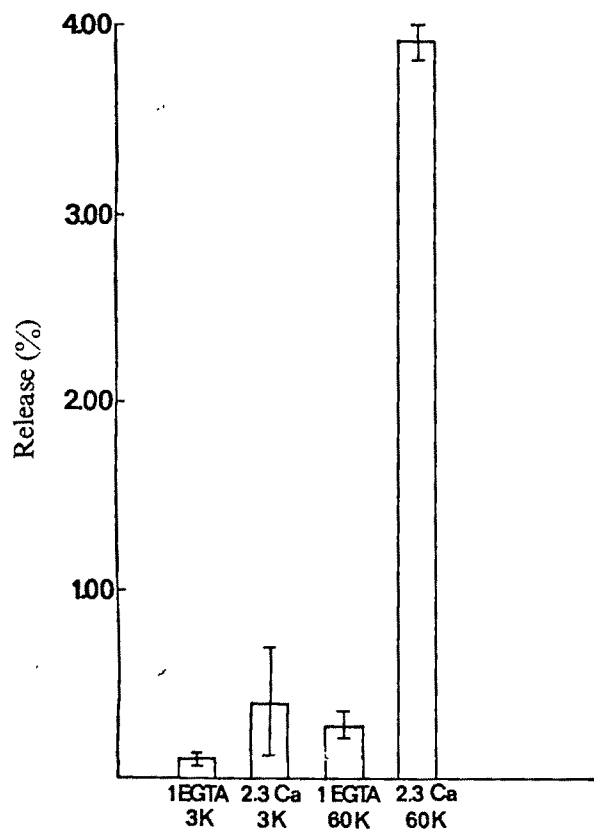


Fig. 1 Release of substance P from superfused synaptosomes. Synaptosomes were placed on filters (Nucleopore N80 CPR, Millipore AP20 prefilter) and superfused for 5 min at 5 ml min^{-1} , thereafter at 10 ml min^{-1} with EGTA+3K (for ionic composition see below). After the baseline efflux had stabilised, pulses of test solutions (see below) were administered for 36 s at 2.4-min intervals in varying sequence. The 2.3 $\text{Ca}+60\text{K}$ pulse was usually given at the end of a run since it released a considerable portion of the substance P available for release (see Fig. 2). Fractions of the superfusates were collected every 18 s into acetic acid. The release studies were carried out at room temperature ($20-23^\circ\text{C}$). At the end of an experimental run the filters retaining the tissue were extracted in 2N acetic acid. Superfusates and filter extracts were lyophilised and the substance P content determined by radioimmunoassay. Each bar in the histogram represents the amount of substance P released during 36 s in response to the respective pulse. The base-line efflux (EGTA+3K) was obtained from the means of the 3 fractions preceding a given pulse. The amount of substance P on filters at time of collection of a given sample was estimated from the amount of substance P on the filter at the end of an experimental run plus the cumulative amount of substance P collected in the effluent. Values are expressed as: amount of substance P collected/estimated amount of substance P on filter at time of collection $\times 100$. Mean \pm s.e.m. ($n=4$; for EGTA+3K, $n=20$; for 2.3 $\text{Ca}+60\text{K}$, $n=6$). Physiological solutions: An artificial cerebrospinal fluid, buffered with HEPES at pH 7.3 and oxygenated with O_2 was used. When necessary, Na^+ was replaced isotonicity by K^+ , and/or Mg^{2+} was replaced by Ca^{2+} . The basic composition ("2.3 $\text{Ca}+3\text{K}$ ") was as follows: 135 mM NaCl, 3 mM KCl, 2.3 mM CaCl_2 , 2.3 mM MgCl_2 , 0.5 mM Na_2HPO_4 , 10 mM glucose, 20 mM HEPES and 0.2% bovine serum albumin. "2.3 $\text{Ca}+60\text{K}$ " contained 60 mM KCl. "EGTA+3K" contained 4.6 mM MgCl_2 and 1 mM EGTA. "EGTA+60 K" contained 4.6 mM MgCl_2 , 1 mM EGTA and 60 mM K^+ .

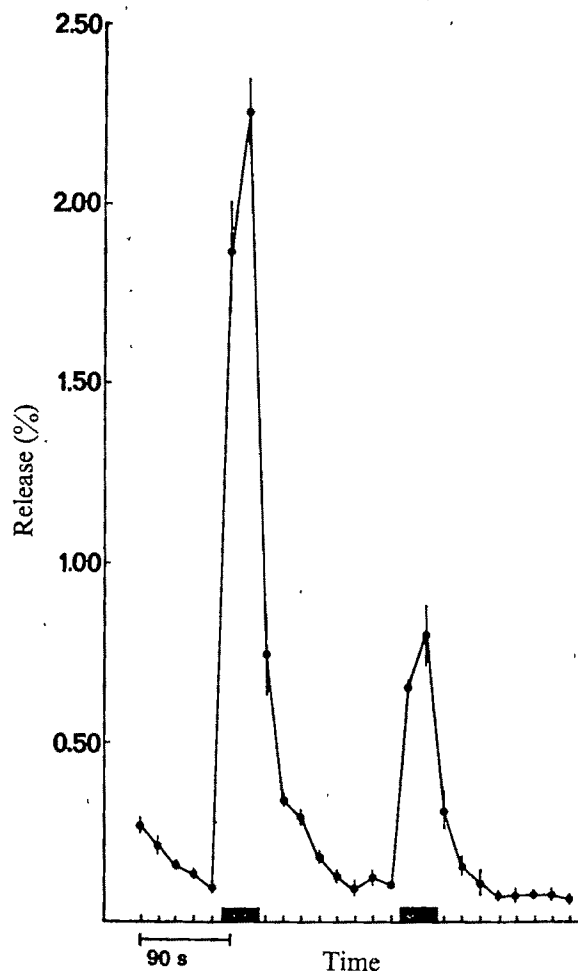


Fig. 2 Time course of release of substance P. Experiments were carried out as described in Fig. 1. Synaptosomes were superfused with EGTA+3K except at bar, where they were exposed to 2.3 $\text{Ca}+60\text{K}$ for 36 s. Each time division is 18 s. Values expressed as: amount of substance P released/amount of substance P on filter at time of collection $\times 100$. Mean \pm s.e.m. ($n=3$).

are therefore confident that the substance P in crude synaptosomal pellets was released from nerve endings and not from any contaminants.

Recently, Otsuka has also reported that the substance-P like immunoreactivity in the perfusate of the isolated spinal cord of new-born rats is increased by soaking the preparation in a solution containing 55 mM K^+ (ref. 11).

These results show qualitatively that substance P can be released in a manner similar to that of other suspected neurotransmitters. Although this finding can be taken to support a transmitter role for substance P, some important quantitative questions remain to be answered. First, the precise amount of substance P needed to mimic the physiological activity of a given synapse, including the time course of that activity, must be determined. Second, the timing and amount of substance P released at that synapse must be determined and shown to be adequate to account for the normal activity of that synapse. Until such quantitative information is obtained the question of whether substance P is a transmitter in the nervous system remains open. In any case, the possibility that substance P may have other functions, such as the modulation of synaptic activity at a pre- or postsynaptic site, should not be neglected.

Since submission of this manuscript, the release of

immunoreactive substance P from an isolated spinal cord preparation of new-born rats²¹ and rat hypothalamic slices²² has been reported.

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Induction of tolerance to the analgesic action of lipotropin C-Fragment

THE C-Fragment of β -lipotropin (lipotropin 61–91), was first discovered as an intact peptide in the pituitary gland^{1,2} and it has recently been shown to be present in brain³. The peptide binds strongly to brain opiate receptors *in vitro*^{4,5} and has potent opiate-like actions on the guinea pig ileum and mouse vas deferens¹⁵. Reports from our laboratory described the potent analgesic properties of C-Fragment when injected into the cerebral ventricles of the cat^{4–6}, and in subsequent studies its analgesic effects have been demonstrated in rodents^{7–9}. The results reported here confirm the analgesic potency of C-Fragment administered intracerebrally in the rat, and show that chronic administration of

the peptide leads to the development of tolerance to its analgesic actions

C-Fragment was isolated from pig pituitary glands as described previously². Cannulae were implanted in the lateral ventricles of male Wistar rats weighing 150–170 g¹⁰. After a recovery period of at least 1 week, response latencies of the animals were measured on a hot plate (54 \pm 0.2 °C) according to Eddy and Leimbach¹¹. The criterion of reaction of the rat was licking of one paw, or intensive jerking with lifting off or jumping on the hind legs. The trial was terminated if response latency exceeded 60 s.

Two experiments were carried out, using the same schedule of chronic peptide administration; groups of 6 rats received intracerebral injections of 4.5 μ g C-Fragment (1.5 μ g μ l⁻¹, dissolved in 1 M NaCl) or of placebo (3 μ l of 1 M NaCl) twice daily (at 9.30 a.m. and 5.30 p.m.) for 2 d

Experiment 1 was designed to detect first the onset of tolerance to the analgesic action of C-Fragment, and second the possible development of cross-tolerance to the action of peripherally-administered morphine. A group of 6 rats was treated with C-Fragment according to the schedule described above. Results from 2 animals were later discarded, owing to improper location of the cannulae. A second group of 6 animals was treated with placebo. The acute analgesic action of the peptide was established by measurement of hot plate response latency before and after the first injection of peptide and placebo.

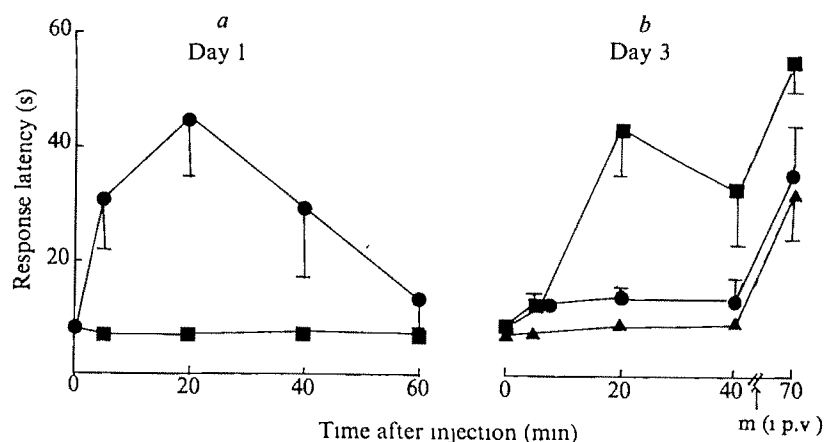
Response latency underwent a large increase in the C-Fragment treated rats (Fig. 1a). The time course of action was fairly rapid; analgesic activity was maximal 20 min after injection, and faded rapidly thereafter. Acute injection of lower doses of the peptide (0.3 and 1.1 μ g) did not significantly increase response latency, indicating that the dose-response curve is rather steep.

At 9.30 a.m. on d 3 of the experiment, both groups of animals received an injection of 4.5 μ g C-Fragment. Hot plate response latencies were monitored both before and after injection, and were compared with corresponding latencies measured for a group of 6 control animals which were given a single acute intracerebral injection of placebo. Forty minutes after the intracerebral injection, the three groups of animals were given a single i.p. injection of 10 mg kg⁻¹ morphine-HCl, and hot plate response latencies were measured 30 min later. The results of these measurements are summarised in Fig. 1(b).

Chronic pretreatment with placebo had no effect on the analgesic response to C-Fragment. The response latencies of animals chronically pretreated with the peptide, however, underwent only a slight increase. This increase was significantly less than that obtained with the placebo-pretreated group ($P < 0.05$, Wilcoxon test). In every case the increases in response latencies of individual animals which had been chronically pretreated with C-Fragment were lower than the increases measured for the same animals after the first injection of the peptide. This indicated that tolerance had developed to the analgesic action of the peptide.

Systemic administration of morphine evoked similar increases in response latency in all three groups of animals (Fig. 1b). There

Fig. 1 Mean response latency as assessed on the hot plate of animals treated intraventricularly with 4.5 μ g C-Fragment twice daily (4 animals, ●) or with placebo twice daily and on d 3 with 4.5 μ g C-Fragment (6 animals, ■). Morphine-HCl (10 mg kg⁻¹) was administered intraperitoneally (i.p.) 40 min after the last injection with C-Fragment. One group of 6 animals (▲) received an intracranial placebo injection on d 3. Results are given as mean \pm s.e.m. Unless otherwise indicated, standard errors are within the size of the points



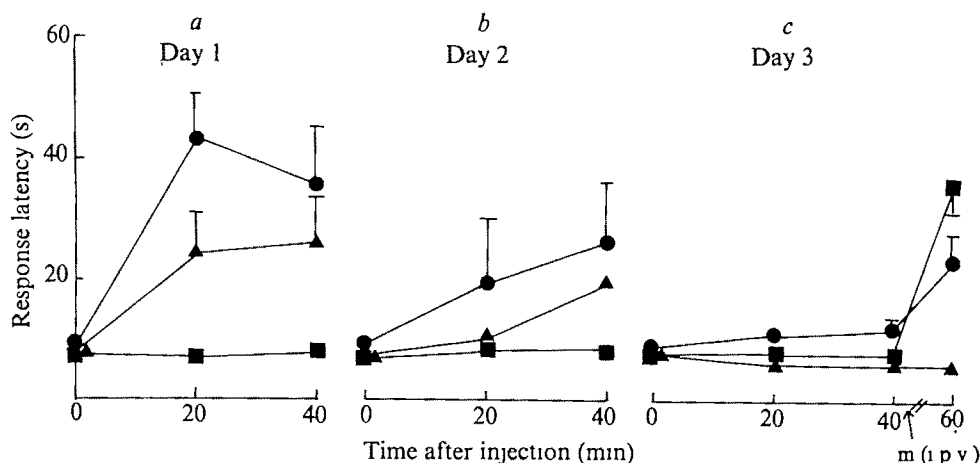


Fig. 2 Mean response latency as assessed on the hot plate of animals treated intraventricularly with 4.5 μ g C-Fragment (5 animals, \bullet) or with placebo (6 animals, \blacksquare) twice daily. One group of 6 animals treated with C-Fragment received Pro-Leu-Gly-NH₂ (1 μ g per animal, subcutaneously) 1 h before C-Fragment on the d 1 only (\blacktriangle). On d 3 morphine-HCl (15 μ g per animal) was administered intraventricularly (m.v.) 40 min after the injection with C-Fragment. Results are given as mean \pm s.e.m. Standard errors are within the size of the points, unless otherwise indicated.

was therefore no sign of cross-tolerance to the narcotic after peripheral administration. However, it is notable that the level of analgesia attained after morphine injection in animals which had been chronically pretreated with placebo followed by a single injection of C-Fragment was substantially greater than that found in the C-Fragment-pretreated or control animals, indicating a probable potentiation of the analgesic effects of morphine by a single dose of the peptide, an effect which seems to disappear after chronic treatment.

Experiment 2 was designed first to examine the possible development of cross-tolerance between the analgesic effects of C-Fragment and intracerebrally administered morphine, and second, to examine the effects of pretreatment with the C-terminal tripeptide of oxytocin, pro-leu-gly-NH₂ (PLG) on the time-course of tolerance development, and on the degree of cross-tolerance developed to morphine. It has been shown previously that pretreatment with oxytocin, or PLG, markedly facilitates the development of morphine tolerance and physical dependence in the rat¹².

Three groups of animals were used in this experiment. Group 1 (5 animals) received a single subcutaneous injection of 1 μ g of PLG dissolved in 0.2 ml saline, and groups 2 and 3 (6 animals) control injections of 0.2 ml saline 1 h before the first injection of C-Fragment. Groups 1 and 2 were given intracerebral injections of 4.5 μ g C-Fragment, and group 3 injections of 1 M NaCl according to the schedule previously described. Hot plate latencies were tested before and after the first and third injections, on d 1 and 2 respectively. On d 3, groups 1 and 2 were again injected with C-Fragment, and group 3 with placebo, and hot plate latencies assessed. 40 min after the final injections of C-Fragment and placebo, all 3 groups of animals were given an intracerebral injection of 15 μ g morphine-HCl. Hot plate latencies were monitored 20 min later. The results of this experiment are summarised in Fig. 2.

Animals pretreated with C-Fragment alone again developed tolerance to the analgesic action of the peptide. This was clearly visible on d 2 (Fig. 2b), and virtually complete on d 3 (Fig. 2c). Pooling the results from experiments 1 and 2, increases in response latencies were significantly smaller on day 3 compared with day 1 both 20 and 40 min after C-Fragment injection ($P < 0.01$, $P < 0.05$ respectively, Wilcoxon signed rank test, 9 animals).

Pretreatment with PLG appeared to exert some depressant effect on the acute antinociceptive action of C-Fragment (Fig. 2a). However, this effect was not statistically significant ($P > 0.1$, Wilcoxon test). Pretreatment with the tripeptide tended to accelerate the development of tolerance to C-Fragment (Fig. 2b and c), but a larger number of animals will be needed to establish this effect with more certainty. However, the PLG-treated animals, which had been chronically injected with C-Fragment showed complete cross-tolerance to the analgesic effect of an intraventricular injection of 15 μ g of morphine-HCl (Fig. 2c). Morphine injection increased mean response latency by 30 s in the placebo-pretreated group, and by 11 s in the group pretreated with C-Fragment alone.

The difference between these values is marginally significant ($P < 0.1$, Wilcoxon test). However, the response latency of the PLG-treated group failed to increase after morphine injection, in any of the animals tested ($P < 0.005$ with respect to both of the other groups). These findings suggest that chronic pretreatment with C-Fragment induces cross-tolerance to morphine. PLG accelerates the development of cross-tolerance and therefore presumably enhances the development of tolerance to the analgesic action of peptide itself.

The results of these studies confirm the high analgesic potency of C-Fragment in the rat. On a molar basis, the analgesic potency of the peptide injected into the lateral ventricle is 30–40 times that of morphine, in good agreement with the findings of Loh *et al.*⁷, and Graf *et al.*⁸. A comparable potency ratio has been reported for opiate-like effects of the peptide after injection into the periaqueductal grey matter¹³.

Chronic administration of C-Fragment leads to the development of tolerance to its analgesic effects, and there are strong indications of the development of some cross-tolerance to intracerebral morphine. The absence of cross-tolerance to peripherally administered morphine must be interpreted with caution, firstly because in animals pretreated with C-Fragment alone, cross-tolerance to intracerebral morphine was not complete, and secondly because the population of receptors involved in mediating the antinociceptive effects of relatively low peripheral doses of morphine may not be identical to that occupied after intracerebral injection¹⁴.

The findings reported here accord with the ability of C-Fragment, and other opiate peptides to induce morphine-like physical dependence¹³, and suggest two intriguing possibilities: that mechanisms involved in the production of tolerance may be important in regulating the actions of endogenous opiate peptides *in vivo*, and that other neuropeptides may play a part in modulating these processes.

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Induction of excessive grooming in the rat by fragments of lipotropin

CURRENT research reveals the existence of endogenous peptides in brain^{1,2} and pituitary tissue^{3,4}, which are presumably derived from lipotropin (LPH) and which have opiate like effects and affinity for opiate receptors (enkephalin¹, endorphins⁵, C-Fragment⁶). N-terminal peptides of ACTH also have measurable, although much lower, affinity for rat brain receptors *in vitro*^{7,8}. ACTH and congeners are known to play a crucial role in the acquisition and maintenance of a variety of behaviours in animals and man^{9,10}. Intraventricular but not systematic administration of these peptides elicits a stretching and yawning syndrome^{11,12}. In rats this syndrome is preceded by a display of excessive grooming^{13,14} and this grooming response can be suppressed by peripheral administration of specific opiate antagonists (naloxone, naltrexone)¹⁵. Morphine also induces grooming¹⁵ and this observation prompted us to study the effect of LPH fragments on excessive grooming in the rat in the absence and presence of an opiate antagonist.

The methodology has been described in detail elsewhere^{14,15}. Briefly, female rats of an inbred Wistar strain (TNO, Zeist, The Netherlands) had plastic canulae implanted in the 3rd cerebral ventricle, 1 week before the observation session. The behavioural procedure consisted of a 15th-s sampling technique, the validity of which has already been established¹⁴. During a 50-min period the observer determined every 15th s whether the rat displayed an element of the maintenance repertoire (that is vibrating, washing, grooming, scratching, licking paw, licking tail)¹⁶. Since the predominant element recorded seemed to be grooming, we prefer to refer to grooming in keeping with previous reports^{11,13,14}. Immediately after intraventricular injections into conscious rats (1 μ l), the rats were placed individually into glass boxes (24 \times 12.5 \times 14 cm) in a low noise room and 15 min later the behavioural analysis began. The effect of naloxone (Endo, Ny, 0.5 ml 100 g⁻¹ subcutaneous 1 mg kg⁻¹) on peptide-induced excessive grooming was studied 25 min after the intraventricular injection of the peptide. Therefore the scoring was interrupted for 5 min to allow for administration of the opiate antagonist, subsequently the behavioural analysis was continued for another 40 min. The following synthetic peptides were tested: ACTH₁₋₂₄, β -MSH (LPH₄₁₋₅₈), ACTH₄₋₁₀ (LPH₄₇₋₅₃), Met-enkephalin (LPH₆₁₋₆₅), Leu-enkephalin ([Leu⁶⁵]LPH₆₁₋₆₅), LPH₆₁₋₆₉, LPH₆₅₋₆₉, α -endorphin (LPH₆₁₋₇₆), C-Fragment (LPH₆₁₋₉₁) was isolated from porcine pituitary glands³.

Saline treated rats, placed in a novel glass box usually display exploratory and grooming behaviour which may last for some 15-20 min after intraventricular injection but then invariably fall asleep and thus show little grooming activity during the observation period, which starts 15 min after the injection. In contrast, the intraventricular injection of 0.3 μ g of LPH₆₁₋₉₁ elicited nearly maximal grooming activity (165 out of 200 possible positive scores). The effect appeared to be dose-dependent. A dose of as little as 10 ng significantly induced grooming activity (Table 1). LPH₆₁₋₉₁ therefore has a higher potency than ACTH₁₋₂₄¹⁴. It should be noted, however, that in contrast to ACTH-induced grooming, administration of LPH₆₁₋₉₁ in low doses such as those used here, not only elicited grooming but also excitation in some

rats typified by quick movements of body and head, jumping, gnawing and body shakes. In a subsequent experiment the effect of subcutaneously administered naloxone on LPH₆₁₋₉₁-induced excessive grooming was studied. Rats treated with 0.1 μ g LPH₆₁₋₉₁ displayed excessive grooming before naloxone (Table 2). However, after subcutaneous administration of 1 mg kg⁻¹ naloxone, excessive grooming was markedly suppressed. Interestingly, no overall behavioural differences were noted between saline/saline and saline/naloxone treated rats, suggesting that naloxone itself did not affect ongoing behaviour. Similar results were obtained using 0.1 mg naloxone and 0.1 μ g LPH₆₁₋₉₁. Structure activity studies were carried out to identify the active amino acid sequence responsible for excessive grooming. It has been found previously that intraventricular injection of sheep β -LPH does not elicit grooming in the rat, unless an extreme dose (2.5 mg) is used¹³. Apparently β -LPH itself is practically devoid of activity in this respect. LPH₆₁₋₇₆ (α -endorphin) was much less active than LPH₆₁₋₉₁. The sequence LPH₆₁₋₆₉ seemed slightly less active than LPH₆₁₋₇₆ (Table 1). LPH₆₁₋₆₅ and [Leu⁶⁵]LPH₆₁₋₆₅ induced hardly any grooming activity over the wide dose range tested (0.1-29 μ g). Similarly, LPH₆₅₋₆₉ was found to be inactive (Table 1). LPH₄₇₋₅₃ which is common to LPH, MSH and ACTH has latent grooming activity¹⁷. Administration of LPH₄₇₋₅₃ in doses up to 40 μ g does not induce excessive grooming (Table 1) whereas administration of LPH₄₇₋₅₀ or [D-Phe⁵⁰]LPH₄₇₋₅₃ (= [D-Phe⁷]ACTH₄₋₁₀) elicits significant grooming at a dose of 1 μ g^{14,15}. As reported previously the sequence LPH₄₁₋₅₈ (= β -MSH) is equipotent to ACTH₁₋₂₄ in inducing the grooming response (Table 1)¹⁴. Excessive grooming was interrupted by short episodes of stretching and yawning only in the case of ACTH₁₋₂₄, LPH₄₁₋₅₈ and LPH₆₁₋₇₆, again suggesting that excessive grooming behaviour and stretching/yawning may result from different mechanisms¹⁴. The results indicate that within the C-fragment LPH₆₁₋₆₉ contains the essential sequence for excessive grooming. Elongation of this sequence, however, markedly enhances the response.

The available data on the affinity of LPH fragments for opiate receptors in rat brain membrane fractions indicate that LPH₆₁₋₉₁ has the highest affinity (IC₅₀ for DHM 2.2×10^{-9} M, for etorphin 3×10^{-7} M^{5,6}) followed by (61-65), (61-69) and (61-76) in that order. The affinity of fragments of ACTH and LPH₄₇₋₅₃ (ACTH₄₋₁₀) studied with labelled DHM is several orders of magnitude lower than those peptides mentioned above and the sequence α -MSH[Ac-Ser³]ACTH₁₋₁₃-NH₂—which is as active as ACTH₁₋₂₄ in inducing excessive grooming¹⁴—is virtually inactive^{7,8}.

Recently, we have reported that subcutaneously administered fragments of ACTH which have some affinity for the brain opiate receptors and which are devoid of *in vivo* corticotropic activity counteract morphine-induced analgesia in the rat as tested on the hot plate^{18,19}.

The biological significance of induction of excessive grooming is unclear. Some authors have interpreted the behavioural signs

Table 1 LPH fragments and excessive grooming

Peptide sequence	Lowest dose at which excessive grooming was observed* (μ g)
LPH ₆₁₋₉₁	0.01
LPH ₆₁₋₇₆	1.7
LPH ₆₁₋₆₉	5.5
LPH ₆₁₋₆₅	>29†
[Leu ⁶⁵]LPH ₆₁₋₆₅	>29†
LPH ₆₅₋₆₉	>20†
LPH ₄₁₋₅₈ (= β -MSH)	0.1
LPH ₄₇₋₅₃ (= ACTH ₄₋₁₀)	>40†
ACTH ₁₋₂₄	0.1

*Grooming was scored using a 15th-s sampling technique (observation session of 50 min, beginning 15 min after intraventricular injection). For all peptides at least 5 dosages were tested with at least 4 rats per group. Saline-treated rats as average reach a score of 20-30 (out of maximum of 200). Significant excessive grooming is observed when scores are above 55.

†No activity observed over a dose range of 0.1 μ g to indicated dose.

Table 2 Effect of naloxone on LPH₆₁₋₉₁ induced excessive grooming in the rat

		Treatment		Number of rats	Amount of grooming	
<i>t</i> = 0 min IIIrd ventricle		<i>t</i> = 25 min subcutaneous			15–25 min	30–70 min
LPH _{61–91}	0.1 µg	saline	0.5 ml	6	32 ± 1*	87 ± 12
LPH _{61–91}	0.1 µg	naloxone	1 mg/kg	6	29 ± 2	6 ± 3
saline	1 µl	naloxone	1 mg/kg	3	14 ± 8	12 ± 7
saline	1 µl	saline	0.5 ml	3	16 ± 6	9 ± 5

*Mean ± s.e.m.

induced by intracranial administration of ACTH-like peptides in terms of sexual excitement^{20,21}. The behavioural response in pigeons has been likened to displacement behaviour²². Both electrical stimulation of limbic structures^{23,24} and central application of Zn²⁺ (ref. 12) may induce grooming activity. The latter observation is of interest in view of the known importance of bivalent cations to the mechanism of action of ACTH^{25,26}.

The present study was aimed at investigating the nature of the peptide-CNS interaction underlying behavioural effects. Since opiate antagonists suppress ACTH and LPH-induced behaviour, one is tempted to conclude that the neural substrate for this behaviour is sensitive to ACTH fragments, LPH fragments and opiates. Interestingly, intraventricular administration of low doses of morphine also induces excessive grooming to the same extent as LPH₆₁₋₇₆¹⁵.

In view of the behavioural effects produced by intracerebral injection of low doses of LPH fragments, the question whether the "opiate-like" activity of these neuropeptides is their most important physiological effect remains to be elucidated.

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Neonatal progesterone and feminine sexual development

It has been generally concluded that the inherent programme of sexual differentiation in both sexes of mammals is female. If androgens are present during the critical periods of sexual differentiation, then both genetic males and females will be organised for masculine reproductive organs¹, hepatic steroidogenic enzymes², hypothalamic control of gonadotropin secretion (tonic)³ and sexual behaviour⁴; whereas an absence of either gonad during the critical developmental periods allows for the expression of the inborn female programme^{1-3,5}. These results have led to the generally held concept that feminine differentiation requires no hormonal imprinting and will occur normally as long as androgens are not present during the critical periods of sexual embryogenesis. We have reported, however, that interference with perinatal pituitary or adrenal function in female rats causes defects in normal pubertal feminine development which suggests that endogenous hormones may be essential for feminine organisation⁶. Unlike oestradiol and testosterone which both masculinise the female rat⁷, progesterone treatment antagonises these effects and protects the developing female from exogenous oestrogens and androgens⁸. In fact, serum progesterone levels in the foetal monkey have been shown to be significantly higher in the female than in the male⁹ and we have recently postulated that perinatal progesterone may be required for feminine neural differentiation¹⁰. We present here evidence demonstrating that neonatal female rats have a markedly higher level of serum and adrenal progesterone than do neonatal males, and since serum progesterone levels can be further increased by exogenous gonadotropins-adrenal (progesterone) axis may be required for normal feminine sexual differentiation.

At 3 d of age the serum progesterone concentration was ten times greater in female pups than in males (Table 1) and the adrenal progesterone concentration was also significantly greater in the neonatal females. Administration of pregnant mare serum gonadotropin (PMSG), which has both follicle stimulating hormone (FSH) and luteinising hormone activities, with FSH predominant¹¹ elevated serum progesterone levels on both 3-d-old male and female rats.

Our progesterone results in neonatal rats are in agreement with findings in adult animals, in that rat adrenals

have been shown to secrete progesterone¹³ and this secretion is selectively enhanced by gonadotropins¹³. Furthermore because the ovary of the rat is steroidogenically inactive^{14,15} and unresponsive to gonadotropins^{16,17} until the second week of life, it seems certain that the neonatal adrenal was the source of serum progesterone, at least in the female. Although the neonatal testis is steroidogenically competent¹⁴, and secretes androgens³⁻⁵, the presence of significant levels of progesterone in the male adrenals suggests that they may also be the source of serum progesterone in the neonatal male.

It now seems possible to describe a functioning sexual neuroendocrine axis in the neonatal female rat. The hypothalamus of the newborn female rat contains increasing levels of gonadotropin releasing hormone (GnRH)¹⁸, and the pituitary of the newborn female rat is more sensitive than that of the male to GnRH, and thus secretes greater quantities of LH in response to GnRH treatment¹⁹. This enhanced sensitivity to GnRH by the female pituitary may be the cause of the much higher levels of serum LH and FSH found in newborn females as compared with males¹⁹⁻²¹. These elevated gonadotropin levels in newborn females may, in turn, stimulate adrenal hormone production resulting in higher serum progesterone levels in the female. There is thus a distinctive sexually dimorphic character in the functioning of the hypothalamic-pituitary-adrenal axis of the neonatal female rat which clearly suggests a role for perinatal hormones in feminine sexual development.

The elevated progesterone levels in the newborn female are of particular importance. During the first few days of life both male and female rats have very high levels of serum oestrogens^{18,21} and androgens²¹, and it is during this period that the brain is most sensitive to the masculinising effects of testosterone and oestradiol⁷. The raised serum progesterone in the newborn female may function as a hormone antagonist and protect the developing female brain from the masculinising actions of the endogenous

oestrogens and androgens and thus, allow for differentiation of the inborn female development pattern. It is also possible, however, that progesterone has a direct effect on female organisation and that feminine differentiation is not a result of an inherent developmental programme, but requires active imprinting by perinatal hormones, such as adrenal progesterone¹⁰.

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Table 1 Progesterone levels in 3-d-old rat pups treated with PMSG

		Serum progesterone (pg ml ⁻¹)	
	No. of pups	Control	PMSG
Male	23	49.9 ± 7.0*	467.9 ± 59.3§
Female	16	444.6 ± 58.6§	604.9 ± 43.1†
		Adrenal progesterone (pg mg ⁻¹)	
		Control	PMSG
Male	21	465.1 ± 48.1	448.6 ± 75.3
Female	12	762.4 ± 107.7‡	603.9 ± 58.4

Within 6 h of parturition half the male and half the female pups in each litter were injected with 16 IU PMSG (NIAMD) and the other half received the diluent, 4 µl of 0.9% NaCl. The same treatment was repeated 24 and 48 h after the first injections. The pups were decapitated on the third day of life, 6-8 h after the third injection. Blood was collected in microcentrifuge tubes and adrenal glands were quickly removed, cleaned, weighed and frozen. None of the serum or adrenals was pooled for the assay, and although adrenal progesterone was determined in duplicate analyses, the small quantities of serum allowed for only single determinations. Progesterone was measured by radioimmunoassay using purchased antibodies (Miles-Yeda) produced by immunising rabbits with progesterone-11α-hemisuccinyl-bovine serum albumin. The progesterone assay was similar to reported procedures²² with the exception that serum and adrenal homogenates were extracted with petroleum ether and the chromatographic step was omitted because the only significant cross reactivity was with 11β-hydroxyprogesterone at only 8%. Recovery from extracted serum using ³H-progesterone ranged from 68 to 73%. The sensitivity of the assay allowed for the detection of 5 pg of progesterone per tube. In ten duplicate determinations the coefficient of variation was 6.3%.

* Mean ± s.e.

Statistical analysis by Student's *t* test comparing control males with control females and controls with PMSG of the same sex.

† *P* < 0.03; ‡ *P* < 0.01; § *P* < 0.001.

Spermatogenesis and 3β-HSDH activity in the testis of the axolotl

THERE has long been considerable debate over the functions of various hormones in promoting or maintaining spermatogenesis. Much of the work in this field has been carried out on mammals, where interpretation is especially difficult because of the heterogeneous content of the seminiferous tubules¹. Although a more favourable situation prevails in some sub-mammalia as lower amphibia^{2,3} and fishes^{4,5} for example, little use seems to have been made of the urodele testis as a model for studies of this type. I present here a short account of spermatogenesis in the axolotl (*Ambystoma mexicanum*), which seems to be particularly suitable for this purpose. In addition to the more usual histological procedures, samples of the tissues were incubated with dehydroepiandrosterone (DHA) or Δ⁵-pregnenolone, in the presence of NAD as carrier and NBT as acceptor for hydrogen (without further staining) in order to demonstrate Δ⁵-3β-hydroxysteroid dehydrogenase (3β-HSDH) activity, according to the method described by Baillie *et al.*⁶.

As in the testis of other urodeles, the early stages of spermatogenesis in *A. mexicanum* occur within the tubules. With the occurrence of the first meiotic divisions, the original tubular form of the testis is replaced by cysts which exhibit a spermatogenic gradient from the periphery to the hilum. Within any one region, however, essentially all the cysts contain germ cells

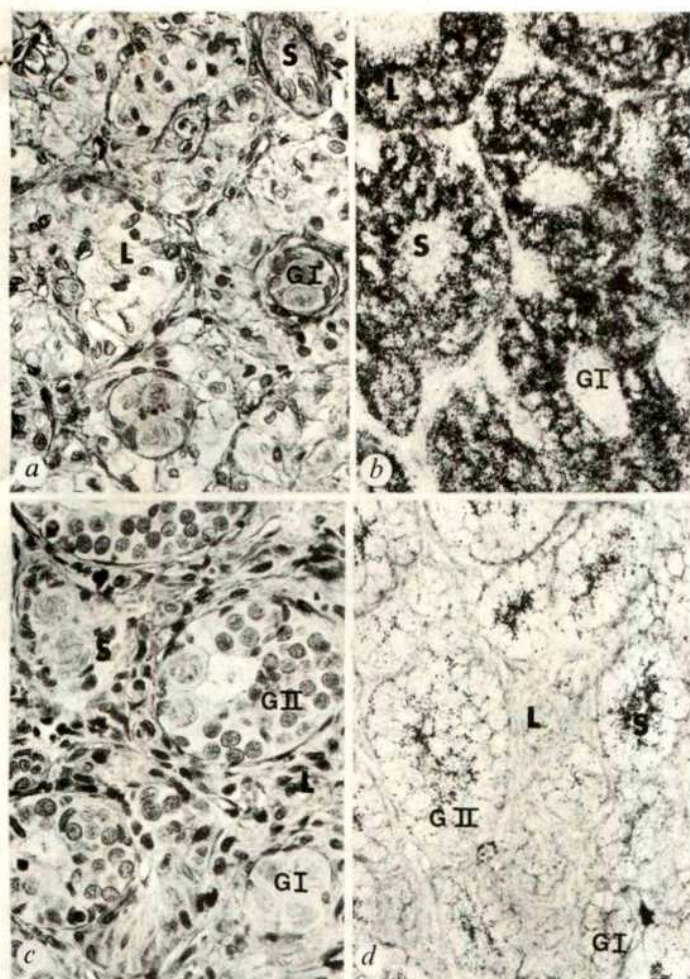


Fig. 1 Correlation between spermatogonial divisions and Δ^5 - 3β -HSDH activity in the glandular tissue. *a*, Interstitial tissue with enclosed nests of primary spermatogonia and Sertoli cells; *b*, demonstration of enzymatic activity restricted to the Leydig cells; *c*, Sertolian tubules with dividing germ cells, surrounded by regressing interstitial Leydig cells; *d*, demonstration of enzymatic activity restricted to the Sertoli cells. L, Leydig cell; S, Sertoli cells; GI, primary spermatogonia; GII, secondary spermatogonia. Magnification $\times 156$.

in the same stage of development. From the young spermatocyte to the late spermatid stage, both Sertoli cells and Leydig cells are relatively rare and the response to any test for 3β -HSDH activity is negative.

During the later stages of spermatogenesis, during spermateliosis, the amount of interstitial tissue begins to increase, a change accompanied by a positive reaction for 3β -HSDH activity. These changes continue until after discharge of the sperm, when large amounts of interstitial tissue envelop the collapsed cysts and there is a relatively massive positive reaction for 3β -HSDH activity. In this 'glandular tissue', characteristic of the urodele testis, small nests of primary spermatogonia accompanied by two or three Sertoli cells, are found between the masses of interstitial cells (Fig. 1*a*). These nests can readily be identified in sections treated for 3β -HSDH activity, where they display a negative response in sharp contrast to that of the enclosing interstitial tissue (Fig. 1*b*).

The Sertoli cells then begin to divide, a process accompanied by the first appearance of the next generation of tubules. As this occurs, the spermatogonia also begin to divide and the amount of interstitial tissue starts to decline (Fig. 1*c*). By this time it is clear that two opposite events are taking place; the emergence of 3β -HSDH activity in the Sertoli cells and its decline in the regressing interstitial tissue. The response of the Sertoli cells to tests of 3β -HSDH activity increases up to the end of the spermatogonial divisions when it ceases (Fig. 1*d*). The

Sertoli cells by then are masked by the newly formed primary spermatocytes and the surrounding interstitium has practically disappeared. The primary spermatocytes then enter the first meiotic division and the next cycle continues as described above.

It can be seen from these results that the structure and general course of events in the testis of *A. mexicanum* make it easier to correlate changes in the Sertoli cells and interstitium with definite stages of spermatogenesis than it is in mammals. Although the study of 3β -HSDH activity only produces a limited amount of information on steroid metabolism by the Sertoli cells and interstitium, it is also interesting to compare briefly the results obtained in the present work with current views about the role of the above cells in mammalian spermatogenesis.

As has been variously claimed for mammals, it seems that axolotl Sertoli cells are capable of steroid metabolism which can, in turn, be linked with spermatogenesis. In mammals, the ability of Sertoli cells to metabolise various substrates to androgens has been most closely linked with meiosis. In the axolotl, however, Sertoli cells 3β -HSDH activity is most closely associated with the spermatogonial divisions. Of particular interest in the axolotl, is that the periods of maximum enzyme activity in the Sertoli cells and the interstitium occur at different times, that is, they are essentially out of phase with each other, a situation not unlike that reported for androgen production by the two cell types in mammals during development⁷. So, although steroid metabolism by the Sertoli cells in the axolotl is seen to be at its height during the early stages of spermatogenesis, 3β -HSDH activity of the interstitium increases rapidly during spermateliosis, reaching a maximum after spermiation. In this animal therefore, the activity of the Leydig cells seems to be related chiefly to the release of the sperm and the development of the secondary sexual characteristics which occurs at this time.

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Rapid increase of foetal corticosteroids after prostaglandin E_2

MATURATION of the foetal hypothalamo-hypophyseal-adrenal axis appears to be involved in the initiation of parturition in several species^{1,2}, but the limiting determinant of this maturational process has not yet been identified. In sheep, an increase in the plasma cortisol concentration of the foetus has been observed before parturition^{3,4} but it has been demonstrated that this increase is not preceded by an elevation in plasma ACTH⁵. It has been suggested that there is a maturation of the foetal adrenal responsiveness to ACTH stimulation^{6,7} and perhaps activation of those enzymes specifically associated with cortisol production⁸. Recently we have shown that a primary prostaglandin, prostaglandin E_2 (PGE_2), is present in the plasma of the foetal lamb and that its concentration increases during late pregnancy⁹. Here we examine the possibility that prostaglandins may be an essential factor in the stimulation of corticosteroid production in the foetus and report a very rapid increase in foetal corticosteroids during PGE_2 infusion into the common carotid artery of foetal lambs.

Foetal catheters were implanted into four animals under

conditions of strict asepsis on days 109, 116, 119 and 120 of pregnancy⁹, and the first infusion was given 15, 10, 7 and 9 days later respectively. Prostaglandin E_2 ($1.6 \mu\text{g min}^{-1}$) was infused in five experiments on four foetuses between days 126 and 133 of pregnancy. Prostaglandin F_{2a} ($1.6 \mu\text{g min}^{-1}$) was infused in three experiments on three foetuses between days 124 and 130 of pregnancy. Infusions were performed on three of these foetuses with a cross-over experimental design allowing a four day recovery period, while the fourth received two infusions of prostaglandin E_2 with the same recovery period. Samples (3 ml) were collected from the femoral artery in three foetuses and from the external jugular vein in one foetus before, during and after the prostaglandin infusion. Foetal heart rate, foetal blood pressure and foetal blood gases were determined as described previously¹⁰. Corticosteroids were measured by radioimmunoassay¹¹ using an antibody prepared against cortisol 21-hemi succinate-BSA, which showed significant cross-reactivity with only corticosterone (10%) and deoxycorticosterone (5%). There was less than 1% cross reactivity with all other steroids tested. The solvent blank was generally less than 10 pg per tube and has not been deducted. The interassay coefficient of variation was about 12%.

Prostaglandins were measured by radioimmunoassay⁹, the mean concentrations of PGE_2 before infusion was 355 ± 78 (s.e.m.) pg ml^{-1} and during infusion of PGE_2 , 2089 ± 841 pg ml^{-1} . The concentration of PGF_{2a} before infusion was 401 ± 54 pg ml^{-1} increasing to 772 ± 128 pg ml^{-1} during infusion of PGF_{2a} .

There was a rapid increase in foetal plasma corticosteroids within 30 min of beginning an intrafetal infusion of PGE_2 (Fig. 1). The concentration of corticosteroids in samples of foetal plasma taken 45 min, 30 min and 1 min before infusion averaged $19\text{--}24 \text{ ng ml}^{-1}$ but increased ($P < 0.01$) to 61 and 62 ng ml^{-1} at 30 and 60 min respectively. The corticosteroids remained raised throughout the subsequent hour of saline infusion, but had fallen to basal levels by the following day. In contrast there was no significant effect of PGF_{2a} infused into the same foetal lambs (Fig. 2). All four lambs were delivered prematurely between 134 and 142 days gestation. This may have been related to the raised cortisol levels resulting from the earlier infusions.

During the control period before infusion of PGE_2 the foetal PaO_2 was 18 ± 1 mm Hg, PaCO_2 51 ± 2 mmHg and pH 7.32 ± 0.01 . The corresponding mean values 30 and 60 min

Fig. 1 Foetal plasma corticosteroid response ($P < 0.01$; mean \pm s.e.m.) to a 1 h infusion of PGE_2 ($1.6 \mu\text{g min}^{-1}$) at $t = 0$ (infusion period = shaded area) for four foetal lambs (gestational age 128 ± 1 d). Saline was infused at the same rate (9.4 ml h^{-1}) during the hour preceding and the hour following the prostaglandin infusion. An analysis of variance for repeated measurements in the same subject was completed for the corticosteroid response to prostaglandin infusion. Tukey Honestly Significant Difference procedure was utilised to test for significance between means when multiple comparisons were made.

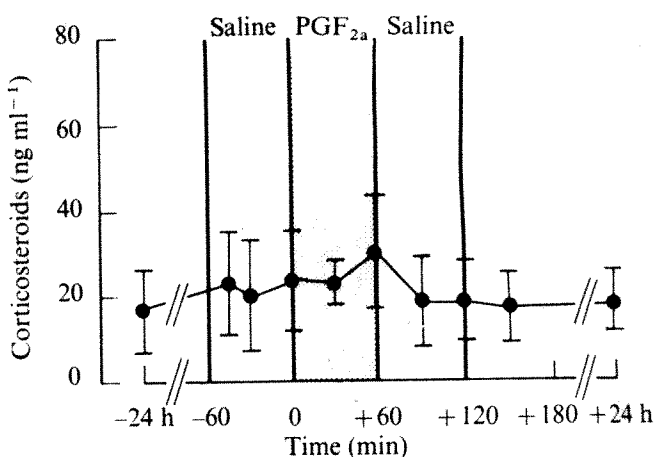
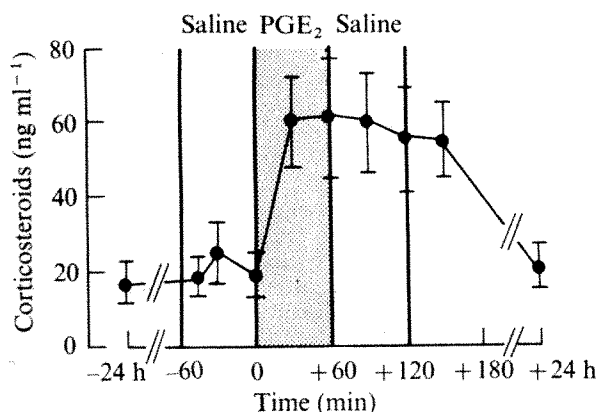


Fig. 2 Foetal plasma corticosteroid response (n.s.; mean \pm s.e.m.) to a 1 h infusion of PGF_{2a} ($1.6 \mu\text{g min}^{-1}$) at $t = 0$ (infusion period = shaded area) in these same foetal lambs (127 ± 2 d). Prostaglandin infusions were administered in a 2×2 cross-over design with a 4-d recovery period allowed between experiments.

after beginning the infusion were PaO_2 18 ± 0.0 and 17 ± 1 mmHg, PaCO_2 52 ± 2 and 51 ± 2 mmHg and pH 7.32 ± 0.004 and 7.32 ± 0.010 . The control values for the PGF_{2a} infusions were PaO_2 21 ± 2 mmHg, PaCO_2 50 ± 3 mmHg, pH 7.31 ± 0.0 and the values at 30 and 60 min after the start of the infusion were PaO_2 20 ± 4 and 21 ± 3 mmHg, PaCO_2 52 ± 1 and 50 ± 1 mmHg and pH 7.30 ± 0.01 and 7.31 ± 0.01 . Foetal heart rate and blood pressure did not change during any of the infusions.

These studies have demonstrated that PGE_2 , infused at a concentration which has no effect on any of the other physiological parameters measured, causes a rapid increase in foetal plasma corticosteroids. Our assay measures principally cortisol and other workers have shown that this is the major corticosteroid produced by the lamb foetal adrenal *in vivo*¹². Infusion of PGF_{2a} , on the other hand, does not alter the plasma corticosteroid concentration of the foetus. This could be due to the lower concentrations of PGF_{2a} achieved during the infusion but it does indicate that the stimulation observed with PGE_2 cannot be attributed to the experimental protocol or sampling regime we have used. The increase in foetal plasma corticosteroids caused by PGE_2 infusion is of importance because it occurred at a time when exogenous ACTH administered in large amounts over 1–2 hours (ref. 7), or endogenous ACTH, released in response to foetal hypoxia¹³ have little effect on foetal plasma corticosteroid concentrations. We have not measured ACTH in the present experiments, but in view of these previous studies, it seems unlikely that the prostaglandin effect is mediated by an increase in ACTH levels. Furthermore, it is unlikely that the present results are attributable to a transfer of maternal cortisol to the foetus, because in the sheep there is little transplacental passage of cortisol, even after raising the maternal plasma cortisol concentration considerably⁷. PGE_2 reduces umbilical blood flow¹⁴ and would not favour cortisol transfer to the foetus. Finally, the concentrations of PGE_2 achieved in the plasma in these experiments are similar to those we have reported at term⁹, thus raising the possibility that circulating PGE may at least augment any effect the trophic hormones may have on steroidogenesis in the foetal adrenal during late pregnancy.

Our results suggest that PGE_2 may exert a direct and specific action on the foetal adrenal gland. Our findings support those of Davies and Ryan¹⁵ who have found that the lamb foetal adrenal possesses those enzymes necessary for cortisol production by day 100–120 of pregnancy, despite the apparent insensitivity of the gland to ACTH at this time. *In vitro* studies with adult adrenal cells have suggested that prostaglandins may be involved in ACTH stimulated steroidogenesis^{13–15}.

The present results raise the possibility that the apparent insensitivity of the foetal adrenal gland to ACTH during much of pregnancy may be associated with an inadequacy of prostaglandin E production. However, our findings are also compatible with the idea that PGE₂ exerts its effect on the adrenal by stimulating the release of pituitary hormones, particularly since we made infusions into the carotid artery.

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Switch from foetal to adult haemoglobin synthesis in normal and hypophysectomised sheep

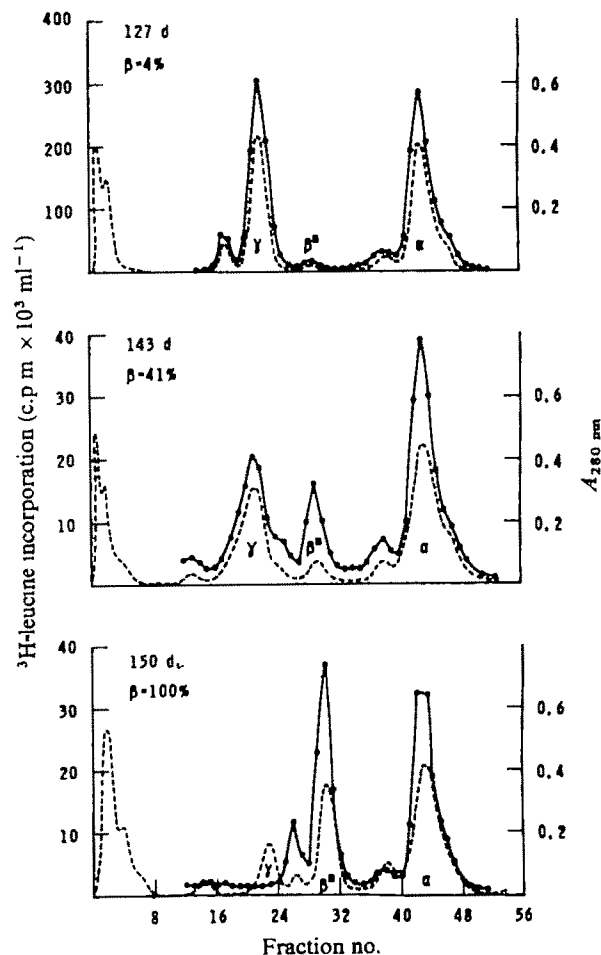
DURING the normal human perinatal period foetal haemoglobin (Hb F; $\alpha_2\gamma_2$) is replaced by the major and minor adult haemoglobins, Hbs A ($\alpha_2\beta_2$) and A₂ ($\alpha_2\delta_2$) respectively^{1,2}. Little is known about the mechanisms involved in the switch from γ - to β - and δ -chain production³ but there is evidence that if the switch could be even partially prevented, so allowing continued production of γ chains into adult life, it might be possible to improve the outlook for patients with the common genetic disorders of β -chain production, particularly sickle-cell anaemia and β thalassaemia⁴. Investigation of the control of human haemoglobin switching has been hampered by the lack of a suitable animal model since none of the small mammals commonly used in the laboratory have a true foetal haemoglobin⁵. However, the developmental changes in the haemoglobins of the sheep broadly mirror those observed in man; two embryonic haemoglobins are replaced early in gestation by a single foetal haemoglobin⁶ which in turn is replaced by the adult haemoglobins at about the time of birth^{6,9}. The two adult haemoglobins of the sheep, Hb A and Hb B, are determined by pairs of alleles for their respective β chains, β^A and β^B . We have examined the switch from foetal to

adult haemoglobin in the sheep to determine whether it is sufficiently similar to that in man to provide a suitable model for studying the control mechanisms involved in haemoglobin switching. In addition, we have carried out preliminary experiments to determine whether the switch may be under hormonal control.

The haemoglobin types of the sheep (Border Leicester \times Suffolk cross) used in this investigation were either AB or BB. A carotid or femoral artery of the foetuses was catheterised between 108 and 124 d gestation⁷, and blood samples were obtained from this time up to birth at 139-144 d and from the newborn lambs for periods up to 1 month post partum. 5 ml blood samples were collected into heparinised syringes and the plasma was removed by centrifugation at 4 °C and stored at -15 °C before radioimmunoassay of cortisol and prolactin^{8,9}. The red cells were used for measurement of globin chain synthesis by incubation with ³H-leucine¹⁰, followed by the separation of the individual globin chains by carboxymethyl-cellulose chromatography¹¹ and determination of the radioactivity incorporated into each chain¹².

The pattern of the globin chain synthesis during development in a normal sheep foetus is shown in Fig. 1. The β

Fig. 1 Globin chain synthesis during development in a catheterised sheep foetus (delivered at 144 d gestation), illustrating the switch from γ - to β -chain synthesis. Red cells from the foetus or the newborn lamb were incubated with ³H-leucine for 1 h in the medium of Lingrel and Borsook¹⁰, in which the AB serum was replaced by saline. The cells were then washed three times with saline, lysed with an equal volume of distilled water and whole cell 'globin' precipitated with acid acetone, without further purification¹². The 'globin' was chromatographed on a carboxymethyl-cellulose column in an 8 M urea 2-mercaptoethanol buffer¹¹ and the chains separated with a 0.0045-0.025 M Na₂HPO₄ gradient (total volume 400 ml; flow rate 30 ml h⁻¹).



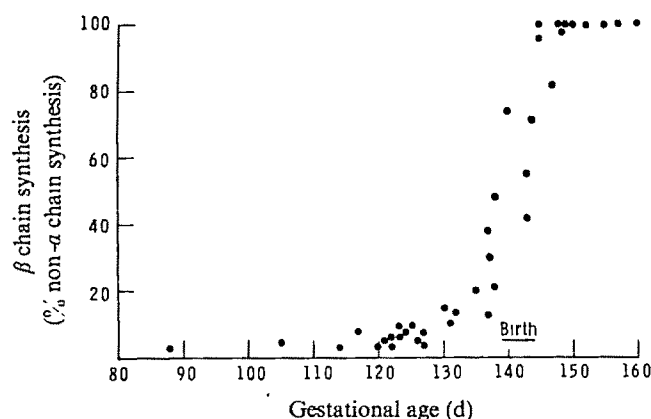
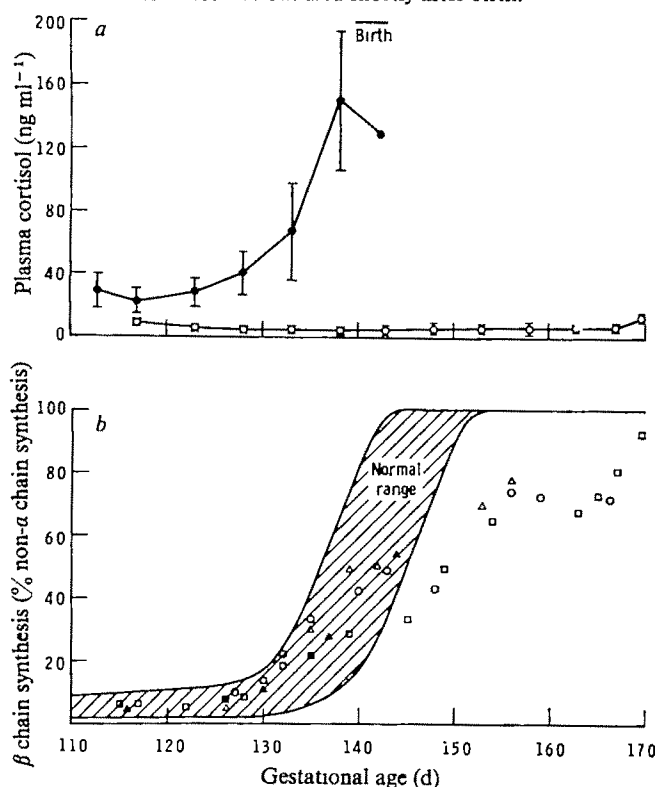


Fig. 2 β -chain synthesis, as a percentage of β - plus γ -chain synthesis, during development in eight catheterised sheep foetuses.

chains of adult Hb in this case were of the β^B type only and at 127 d gestation accounted for approximately 4% of the total non- α chains synthesised. By 143 d, however, the proportion had risen to 43% and by 150 d the switchover was complete and β -chain synthesis had totally replaced γ -chain synthesis. The combined data from eight normal foetuses are shown in Fig. 2. A low level of β -chain synthesis can be detected at early stages of gestation, as is the case in man¹³⁻¹⁵. The increase in β -chain synthesis is first seen at about 130 d gestation and replacement of γ -chain synthesis is virtually complete by 145–150 d. The whole process therefore takes only 2–3 weeks and is completed within a few days after birth, confirming a previous report⁶ that the switch in sheep is considerably more rapid than that in man.

Although little is known about the control of the changes in haemoglobin synthesis during development, the involve-

Fig. 3 *a*, Mean plasma cortisol levels in seven normal (●) and three hypophysectomised (○) foetuses, \pm standard error of the mean. *b*, Percentage β -chain synthesis in the three hypophysectomised foetuses compared with the normal range taken from Fig. 2. □, Hypophysectomised 114 d, delivered 173 d. △, Hypophysectomised 115 d, delivered 158 d. ○, Hypophysectomised 124 d, delivered 171 d. All hypophysectomised foetuses were delivered live but died shortly after birth.



ment of a humoral factor has been implicated by the findings of increased maternal Hb F production during early pregnancy^{16,17} and by the apparent synchrony of the switch in different erythropoietic organs¹⁸. For these reasons we have examined the effects of hypophysectomy on the relative rates of Hb F and Hb A production in the developing foetal lamb. Previous experiments have shown that in the absence of the pituitary, adrenal development does not occur, parturition is prevented, and the foetus continues to grow *in utero* past term¹⁸.

Three foetuses were hypophysectomised by coagulation diathermy¹⁸ at 114, 115 and 124 d gestation. The evidence that this was carried out successfully is based on the measurement of cortisol and prolactin levels in foetal plasma, as well as subsequent histological examination of the pituitary fossa. Plasma cortisol levels in the normal and hypophysectomised foetuses are shown in Fig. 3*a*. Clearly the sharp increase in cortisol levels which normally occurs a few days before birth has been abolished in the hypophysectomised foetuses, where the amounts remained at <20 ng ml⁻¹ until the pregnancy was terminated. Prolactin levels also remained low in the hypophysectomised foetuses as compared with the normals (I. C. McMillen *et al.*, unpublished). The birth weights of the three foetuses were 5.1 kg, 7.0 kg and 8.3 kg after 158, 171 and 173 d gestation respectively. The range for the normal newborn sheep at term was 3.5–6.5 kg. Measurements of packed cell volume and pO_2 in the hypophysectomised foetuses remained within normal limits throughout the post 'term' intrauterine period.

The pattern of the haemoglobin switch in the hypophysectomised foetuses is shown in Fig. 3*b*. An increase in β -chain production was observed at about the same time as that seen in the normal foetuses but the rate of increase was much reduced. The changeover to β -chain production, however, went almost to completion in two of the foetuses that survived to 170 d.

These studies suggest that the chronically catheterised sheep foetus provides an excellent model for examining the switch from foetal to adult haemoglobin production. The data from the eight normal foetuses examined here indicate that the switch is confined to a short period prior to birth and, once activated, proceeds extremely rapidly. In hypophysectomised animals the switch appears to begin at the same time as in normal animals but then proceeds more slowly. This suggests that the initial event in increasing β -chain synthesis may be independent of an intact pituitary but that the process is accelerated in its presence. Previous work (reviewed in ref. 19) has demonstrated the importance of the pituitary–adrenal axis and the production of cortisol in preparing the foetal lamb for extrauterine life. Clearly this preparation includes the replacement of foetal haemoglobin by the adult form but the precise role of the pituitary–adrenal axis remains to be determined.

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Changes in phosphorylation of P light chain of myosin in perfused rabbit heart

It is now well established that myosins from vertebrate skeletal and smooth muscles possess a light chain component of 18,000 to 20,000 molecular weight, the P light chain, that can be phosphorylated by an enzyme present in sarcoplasm¹⁻³. This enzyme, myosin light chain kinase, of molecular weight about 77,000 (ref. 4 and E. V. Pires and S.V.P., unpublished) is highly specific for the transfer of the γ phosphate of ATP to a single serine residue¹ close to the N terminus of the P light chain⁴. Fully phosphorylated myosin therefore contains 2 mol covalently bound P mol⁻¹ as two P light chains are present in each myosin molecule. Dephosphorylation of the P light chain is catalysed by an enzyme, myosin light chain phosphatase, that has recently been characterised⁵ and shown to be highly specific for the P light chain. Myosin light chain phosphatase has a molecular weight of 70,000 and is also present in the sarcoplasm. The presence of such a highly specific enzyme system for the phosphorylation and dephosphorylation of the P light chain strongly suggests a role in the physiological function of muscle. The amounts of the two enzymes present in skeletal muscle are such that it is unlikely that a complete cycle of phosphorylation and dephosphorylation of the P light chain can occur during a single twitch^{3,7}; it could, however, take place within a matter of seconds. We report here that changes in the phosphorylation of the P light chain in the perfused rabbit heart can be correlated with changes in the physiological state of the muscle.

Attempts to demonstrate differences in the enzymic

behaviour of myosin as a consequence of phosphorylation have been unsuccessful with the protein isolated from white skeletal muscle⁸, although there are reports that the low level of ATPase activity of actomyosin from smooth muscle can be increased by phosphorylation of the P light chain⁴. A possible indication of the role of phosphorylation of the P light chain is given by comparison of the myosin light chain kinase and phosphatase activities of different types of rabbit muscle³. Whereas the phosphatase activity is similar per gram wet weight in all muscle types so far studied, the kinase activities are much greater in skeletal than in cardiac and smooth muscles. The enzymic data suggest that if both enzymes are fully active in fast skeletal muscle the P light chain would be in the phosphorylated rather than the dephosphorylated state. On the other hand, in smooth and cardiac muscle, where the enzymic levels are more evenly balanced, changes in phosphorylation of the P light chain might be more readily detected in physiological conditions. We have, therefore, investigated changes in the state of phosphorylation of the P light chain in the perfused rabbit heart. These studies are complementary to those reported on the phosphorylation of troponin I⁹ and in most cases, the analyses of the P light chains were carried out on the extracts obtained after the isolation of troponin I from the hearts used in this study⁹. When the light chain fraction of myosin alone was isolated the methods used for perfusion, measurement of force and homogenisation of the hearts were identical with those described in detail elsewhere (ref. 9 and R.J.S., S.V.P. and C. M. Roberts, unpublished). In these cases the affinity chromatographic step for the isolation of troponin I was omitted.

When the hearts were removed from rabbits under anaesthesia by freezing *in situ* with a Wollenberg clamp the myosin was fully phosphorylated. The P light chains of myosin isolated immediately after death from the hearts of rabbits killed by stunning were only partially phosphorylated. If, however, the hearts from animals killed by stunning were perfused for 10-15 min with modified Krebs Henseleit buffer (ref. 9 and R.J.S., S.V.P. and C. M. Roberts, unpublished) the phosphate content of the myosin returned to 2 mol mol⁻¹; that is, the light chain fraction was fully phosphorylated (Table 1).

Adrenaline was added to the perfusion medium in the range 0.2-4.0 μ M and the light chains isolated from the homogenate prepared when the increase in force was maximal (usually 20-30 s after adrenaline addition) as described in the experiments in which the effect of adrenaline on the level of phosphorylation of troponin I was studied⁹. The phosphate content of the P light chain fell during the inotropic response, but although the

Table 1 Effect of procedure used for isolation of the rabbit heart on the level of phosphorylation of the P light chain of cardiac myosin

Procedure	No. of determinations	P content of myosin (mol mol ⁻¹)*
Excised and homogenised after death by stunning	5	1.0 \pm 0.28
Anaesthetised with nembutal, heart homogenised after freezing <i>in situ</i>	3	2.2 \pm 0.20
Killed by stunning: perfused 15 min	7	2.0 \pm 0.19
Killed by stunning: perfused 100 min	3	2.1 \pm 0.07

Ventricles were homogenised in 8 M urea, 75 mM Tris, 45 mM HCl, 1 mM CaCl₂, 15 mM 2-mercaptoethanol, pH 8.0 (ref. 9) and troponin I was removed by affinity chromatography^{9,10}. The bulk of the protein in the eluate was precipitated by an equal volume of ethanol and a crude light chain fraction was precipitated from the supernate by 10% (w/v) trichloroacetic acid. The precipitate was dissolved in 0.5 ml 1.0 M NaOH, diluted with 25 ml of 4 M urea, 50 mM Tris, 30 mM HCl, 15 mM 2-mercaptoethanol, pH 8.0, applied to a DEAE cellulose column and washed in with the same buffer containing 32 mM KCl. The cardiac light chains were eluted with the buffer containing 83 mM KCl, precipitated with 10% trichloroacetic acid and washed with 5% trichloroacetic acid before analysis for nitrogen and phosphorus¹¹. The proportion of the total protein in the fraction represented by the P light chain was estimated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis at pH 7.0; (ref. 1) relative amounts of protein in each band were determined for each estimation by a dye elution technique¹².

* Assuming 2 mol P light chain per mol myosin¹; \pm standard error of mean.

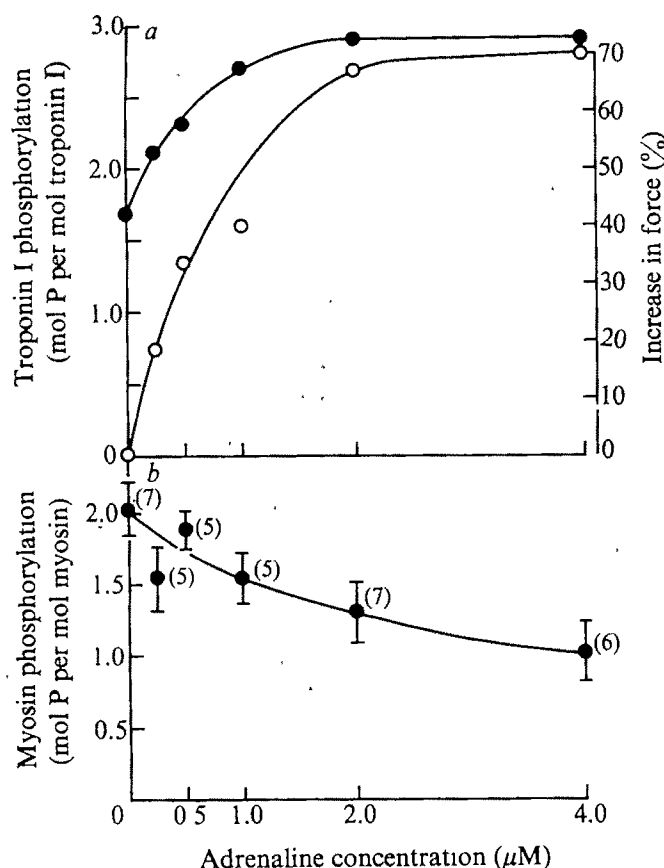


Fig. 1 Phosphorylation of the P light chain of myosin and of troponin I associated with the inotropic response of the perfused rabbit heart to adrenaline. See text for experimental conditions. Number of experiments carried out indicated in parenthesis. *a*, Changes in phosphorylation of troponin I and the increase in force developed on addition of adrenaline to the perfusate. From Solaro *et al.*^{9,11}. *b*, Changes in phosphorylation of the P light chain of myosin on addition of adrenaline. Most determinations were carried out on the same extracts as used in the experiments illustrated in *a*.

maximum contractile response was obtained at 2 μM adrenaline, the phosphate content continued to fall with increasing adrenaline concentration reaching 1.1 ± 0.20 mol per mol of myosin at 4 μM adrenaline, that is, about 50% of the control value (Fig. 1). If perfusion was continued for 5–10 min after the inotropic response, by which time force had returned to the level obtained before adrenaline addition, the phosphate content of the P light chain fraction returned to the original value, 2.0 mol per mol of myosin.

A similar increase in contractile force to that obtained with 2–4 μM adrenaline could be obtained by reducing the Na⁺ concentration from 144 mM to 55 mM and increasing the Ca²⁺ concentration of the perfusion medium from the normal value of 2.5 mM to 7.5 mM. When the light chain fraction was isolated at maximum response as in the previous experiments the phosphate content fell to a value equivalent to 0.8 ± 0.07 mol per mol of myosin. Under these conditions there was no change in the troponin I bound phosphate which remained at the control value of 1.6 mol mol^{-1} (ref. 9).

When Ca²⁺ was excluded from the perfusion medium the hearts stopped beating after 30 s at which moment the phosphate content of the P light chain corresponded to 1.3 ± 0.13 mol per mol myosin.

The findings reported represent the first demonstration of a change in the state of phosphorylation of the P light chain of myosin that apparently correlates with a change

in physiological response of muscle. In both cases in which the force developed by the myocardium increased, dephosphorylation of the P light chain occurred.

The two forms of intervention employed to increase force production in the myocardium, that is, increasing Ca²⁺ and lowering Na⁺ concentrations, and adding adrenaline to the perfusate would both be expected to increase the Ca²⁺ concentration within the cell. Such a change in internal Ca²⁺ concentration should favour phosphorylation of the P light chain for myosin light chain kinase requires this cation for activity¹. The results reported indicate that the reverse is the case as net dephosphorylation of the P light chain takes place under both conditions. In contrast, on adrenaline treatment the activation of 3':5'-cyclic AMP-dependent protein kinase leads to the expected increased phosphorylation of troponin I². Thus when the myocardium responds to adrenaline by increased force production, net phosphorylation and dephosphorylation occur simultaneously at specific sites on the contractile and regulatory proteins of the functioning myofibril. If the contractile force is raised by decreasing the Na⁺ and increasing the Ca²⁺ concentrations in the perfusion medium, however, only the phosphorylation level of the P light chain appears to change.

Without further evidence it would be presumptive to conclude at this stage that dephosphorylation of the P light chain is directly related to the increased force developed by the myocardium. Nevertheless evidence suggesting involvement of the P light chain of myosin in the interaction with actin is beginning to accumulate and phosphorylation of this component might be expected to modify that interaction³. The analytical data available so far suggest that under the conditions studied, the increase in force in the myocardium is accompanied by a change in phosphorylation of the myosin from 2 to 1 mol per mol of myosin. If further investigation confirms that this is the case, the data could be interpreted to imply that only one of the light chains of each myosin molecule is dephosphorylated. Such an interpretation is intriguing and raises the question of the role of the two heads of the myosin molecule, in which the P light chains are located, and the function of phosphorylation in modulating possible cooperative effects associated with the heads.

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Directed selective pressure on a β -lactamase to analyse molecular changes involved in development of enzyme function

THE study of protein mutants has proved a powerful technique in the elucidation of structure-function relationships (see for example refs 1 and 2), but this approach has hardly been used strategically in the investigation of enzyme mechanisms, though its potential for clarifying the role of individual amino acids and their effect on catalytic rates is obvious. We report here the consequences of directed selective pressure on the plasmid-coded β -lactamase from *Escherichia coli* RTEM. Selection for strains with increased resistance to cephalosporin C has allowed the isolation of mutant enzymes whose catalytic properties are changed in the desired direction.

To trace the evolutionary improvement of an enzyme in molecular terms and to watch the stepwise development of its catalytic apparatus, we need an enzyme susceptible to directed selective pressure towards an altered catalytic function. So that mutational changes may be fully interpretable, the tertiary structure must be known. These criteria are fulfilled by the plasmid β -lactamase from *E. coli* RTEM. The enzyme is a monomer (molecular weight approximately 27,000), its sequence is almost complete (R. Ambler, private communication), its crystal structure has been solved to 5.5 Å (ref. 3) and work at higher resolution promises well (J. R. Knox, private communication). Although the directed selection of mutant bacterial enzymes has been achieved previously⁴⁻⁸ and mutant β -lactamases with altered substrate activity spectra have been observed⁹; in no case, unfortunately, is the tertiary structure of the relevant enzyme available, and mechanistic interpretation of the observed amino acid changes has not been possible.

Since the β -lactamase from *E. coli* RTEM hydrolyses benzylpenicillin 30 times faster than cephalosporin C, and since cephalosporin C has reasonable antibiotic activity against Gram-negative bacteria, mutagenesis was followed by selection for strains with increased resistance to cephalosporin C. (Superficially, any extension of the range of antibiotic resistance encoded by a bacterial plasmid could create a biohazard. However, such species already exist, and cephalosporin C-resistant strains (for example, of *Haemophilus*) have been isolated in the clinical laboratory. At least 10% of plasmid-coded resistant enteric species

harbour a plasmid which produces a lactamase active on cephalosporin C, and it is evident that the current experiments will not produce anything that does not already have its counterpart in nature (S. Falkow, private communication; see also ref. 10). Indeed, the studies initiated may usefully contribute to our understanding of the basis of such plasmid-mediated resistance.) Because not all resistant colonies will have a more effective β -lactamase (some colonies may survive by lowering the accessibility of the antibiotic to the killing loci, and others may contain mutations in the killing loci proteins themselves), a second selection was devised to eliminate all colonies whose survival was not derived from a mutation in the plasmid. The plasmids from 415 mutant survivors were transferred to a cephalosporin C-sensitive nalidixic acid-resistant female strain, which was then challenged with cephalosporin C and the donor males eliminated with nalidixic acid. To ensure that all mutated plasmids were screened, transfer was done on plates. (Since plasmids are derepressed for further transfer after initial mating¹¹, transfer in broth immediately after mutagenesis could lead to the first-transferred plasmids "taking over" the culture.) The efficiency of plasmid transfer was 100%. In this way only those colonies containing a favourably mutated plasmid were isolated.

Of the lysates from cultures of the 77 colonies that survived the second selection, four strains had the same β -lactamase specificity as the wild type but appeared to contain much higher levels of enzyme, one strain contained a β -lactamase of altered substrate specificity, and one strain showed changes in both activity and substrate specificity (see Table 1). Lysates from the remaining cultures showed no significant differences from the wild type, indicating a facile mechanism for the emergence of plasmid-borne resistance to cephalosporin C that does not involve the β -lactamase. One obvious possibility is that the mutated plasmid codes for a protein that lowers cell permeability to the antibiotic¹².

To distinguish more precisely between strains that produce more of the wild-type enzyme and strains that produce enzyme with a higher k_{cat} , the crude lysates were titrated with rabbit anti-(wild type)- β -lactamase antibody¹³. This showed that strain h2, for instance, produces 20-30 times more enzyme of unchanged specificity (Table 1). Strains h3, h4 and h6 behave similarly. Mutagenesis evidently most readily leads to super-producers of wild-type β -lactamase. In contrast, strain h1 produces 11-18 times more β -lactamase than the wild type, but this enzyme has altered V_{max} values (Table 1). The purified h1 enzyme and

Table 1 β -lactamase activity of mutant strains of *E. coli* RTEM*

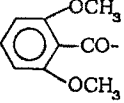
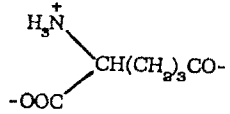
Mutant	Mutagen	Activity in crude extract†		Relative V_{max} ‡	
		Benzylpenicillin	Cephalosporin C	Benzylpenicillin	Cephalosporin C
h1	NTG	5.6	22	0.3	2
h2	EMS	22	31	1	1
h3	EMS	10	10	0.8	—
h4	EMS	10	14	0.6	—
h5	EMS	0.4	2.5	0.5	2
h6	NTG	2.5	2.5	1	1

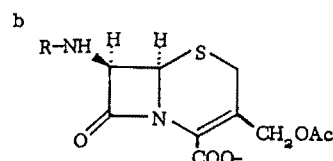
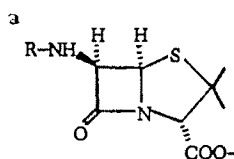
**E. coli* W3310 containing the plasmid RTEM was provided by Dr K. Sargeant of the Microbiological Research Establishment, Porton. Cultures were grown in 1% CY medium¹⁴. Mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) was done¹⁵ at 100 μ g ml⁻¹ for 30 min. Mutagenesis with ethyl methane sulphonate (EMS) was done at 20 μ l ml⁻¹ for 90 min. Challenge with cephalosporin C was done on agar plates with antibiotic concentrations of 0.75, 1, 2, and 3 mg ml⁻¹. Surviving colonies after replica plating (to eliminate colonies surviving by virtue of antibiotic depletion) were transferred to a lawn of female *E. coli* C600, Leu⁻, Thr⁻, Nal^r (provided by Professor F. Ausubel), incubated for 90 min, then covered with soft agar containing cephalosporin C (400 μ g ml⁻¹) and nalidixic acid (20 μ g ml⁻¹). Survivors were purified twice on plates containing cephalosporin C (200 μ g ml⁻¹) and nalidixic acid (20 μ g ml⁻¹).

†Relative to a crude extract of the wild-type strain grown under identical conditions. The supernatant after lysis in the French press was used. Microiodimetric^{17,18} and ultraviolet¹⁹ assays were used.

‡Relative to the wild-type strain. Derived from the gradient of the antibody titrations, in which increasing amounts of rabbit anti-(wild type)- β -lactamase antiserum were added to a fixed number of units of β -lactamase activity. Residual enzyme activity was assayed after 15 min.

Table 2 Relative V_{max} values for penicillin (I) and cephalosporin (II) analogues with the purified enzyme from the wild type (WT) and from mutant h1

R:	H—	$\psi\text{CH}_2\text{CO—}$	$\psi\text{CH}(\text{NH}_2^+)\text{CO—}$		
penicillin analogue: $\frac{V_{max}^{h1}}{V_{max}^{WT}} (x)$	0.12	0.3	0.3	0.1	—
cephalosporin analogue: $\frac{V_{max}^{h1}}{V_{max}^{WT}} (y)$	11	0.75	0.16	3.5	1.4
specificity change (y/x)	90	2.5	0.5	35	—



the wild-type enzyme¹⁴ were both homogeneous on gel electrophoresis and isoelectric focusing, with no difference in the pI values, and no major changes in the tryptic peptide maps. Yet h1 is undoubtedly a true mutant (the LD_{50} with cephalosporin C for the wild type is $5 \mu\text{g ml}^{-1}$, and for h1 is $95 \mu\text{g ml}^{-1}$), and the h1 β -lactamase has catalytic properties altered in the expected direction (see Table 2). Strain h1 is a double mutant, producing more of a modified enzyme. (Double mutants are common when NTG is used¹⁸.)

From Table 2, it is evident that the h1 β -lactamase is, in V_{max} terms, 1.4-fold more effective in hydrolysing cephalosporin C (the K_m is also halved), and 3-fold less effective in the hydrolysis of benzylpenicillin (K_m unchanged). There is a more dramatic change when the unacylated β -lactam nuclei are compared: with 7-aminocephalosporanic acid, the h1 enzyme is more than 10 times more effective, yet with 6-aminopenicillanic acid the h1 enzyme is eight times less effective than the wild-type enzyme.

We have therefore achieved the desired alteration in the catalytic properties of the enzyme. The molecular basis of the changes in the catalytic effectiveness of this and other derived mutant enzymes is being investigated.

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Polypeptide elongation factor 1 and the control of elongation rate in rat liver *in vivo*

THE development of rapid kinetic methods for measurement of elongation rate in protein synthesis *in vivo*, independent of initiation rate, has made possible the study of the control of elongation in relation to changes in overall protein synthesis^{1–4}. In rat liver a 40% reduction in elongation rate (from about six to four amino acid residues per ribosome) is associated with thyroparathyroidectomy⁴; the normal rate is restored by triiodothyronine injections⁴. The work reported here concerns the role of polypeptide elongation factor 1 (EF1), the factor responsible for aminoacyl-tRNA binding to ribosomes, in the control of elongation rate in this system. Several studies have suggested that EF1 may have regulatory significance in eukaryotic systems⁵, however no direct correlation between EF1 activity and elongation rate *in vivo* has previously been described.

Male Long-Evans rats, aged 2 months, were used in all experiments. Conditions for surgery and animal maintenance have been described². Livers were quickly excised from decapitated animals and homogenised using a loose-fitting Dounce homogeniser in 3 vol of cold Medium A (0.25 M sucrose, 0.05 M Tris-Cl, pH 7.5, 25 mM KCl, 5 mM MgCl_2 , 5 mM 2-mercaptoethanol). Post-ribosomal supernatant fractions (S-100) were obtained by centrifugation at 15,000g for 10 min and 100,000g for 90 min at 4°C. Polyribosome fractions were obtained from the 15,000g supernatant fraction by sedimentation for 4 h at 240,000g through a layer of 2 M sucrose containing the other ingredients of Medium A. About 40% of liver tRNA was recovered in the 240,000g pellet. For direct assay of bound EF1, polyribosome pellets were gently resuspended in Medium A at 20–30 A_{260} units ml^{-1} . Contrary to another report⁶, no increase in EF1 activity was obtained on

Table 1 Elongation factor 1 activity in S-100 fractions from livers of normal, thyroidectomised, and triiodothyronine-treated rats

Group	Average body weight (g)	Growth rate (final 7 days) (g d ⁻¹)	Binding activity* (Units per mg protein)	Polymerisation activity† (Units per mg protein)
Normal	218	6.2±1.4	127±16 (26)	53±4 (20)
Thyroidectomized	155	2.1±2.3	126±18 (14)	55±5 (14)
T3-treated	187	n.d.	139±17 (10)	n.d.

*1 unit = 1 pmol ³H-phenylalanyl-tRNA bound min⁻¹ at 30 °C in a system containing 30 mM Tris-HCl, pH 7.5, 120 mM NH₄Cl, 5 mM MgCl₂, 4 mM 2-mercaptoethanol, 25 µg poly(U) acid (Miles Laboratories), 0.4 mM GTP, 2 A₂₆₀ units puromycin-treated rat liver ribosomes, 18 pmol (16,000 c.p.m.) ³H-phenylalanyl-tRNA and 1–5 µg S-100 protein in a final volume of 0.12 ml. After reaction for 5 min, binding was assayed on nitrocellulose filters¹⁴. Results are presented as mean±s.e.d. (number of animals). Each preparation was assayed 3–5 times. Polymerisation activity was <10% on the basis of recovery of phenylalanine-containing peptides by paper chromatography¹⁵ and on filter paper disks⁶ washed in hot trichloroacetic acid. Protein was estimated by the method of Schaffner and Weissman¹⁴.

†1 unit = 1 pmol ³H-phenylalanyl-tRNA incorporated min⁻¹ at 30 °C in a system containing 50 mM Tris-HCl, pH 7.5, 80 mM NH₄Cl, 4 mM MgCl₂, 4 mM dithiothreitol, 25 µg poly(U), 0.4 mM GTP, 0.5 A₂₆₀ unit puromycin-treated rat liver ribosomes, 20 µg EF2 protein, 16 pmol ³H-phenylalanyl-tRNA and 2–10 µg S-100 protein in a final volume of 0.12 ml. Incorporation into polyphenylalanine after 0 min reaction was determined on filter paper disks⁷.

n.d., not determined.

substitution of 1% Triton X-100 or Brij-58 for 0.5% deoxycholate. Bound EF1 was released by treatment of polyribosomes with NH₄Cl at 0.5 M (salt washing). EF1-dependent binding of aminoacyl-tRNA to ribosomes was assayed in a polyuridylic acid system¹⁰, using rat liver ribosomes isolated by sedimentation at 100,000g for 2 h and treated with puromycin for release of endogenous mRNA and peptidyl-tRNA¹¹. Polymerisation of polyphenylalanine was measured in a similar system containing an excess of EF1-free EF2 prepared by Sepharose 4B chromatography¹⁰, or a saturating level of EF1 purified by CM-Sephadex chromatography¹² and free of EF2. Rat liver transfer RNA charged with ³H-L-phenylalanine (New England Nuclear) was prepared by standard procedures¹³.

Table 1 summarises the results of EF1 assay for S-100 fractions of liver from normal (including sham-operated) and thyroidectomised rats, as well as animals given excess hormone (40 µg per 100 g body weight) for 3 d before experiment. No significant differences in EF1 activity were obtained by either assay. Time and concentration dependencies in the assays also were identical for the three groups. This activity therefore does not correlate with the changes of elongation rate found in these animal groups *in vivo*^{2,4}. The result contrasts with that in temperature-acclimated toadfish where soluble EF1 levels and elongation rate in liver show coordinate changes during metabolic compensation for temperature change^{1,5,17}. The difference may be due to an excess of the binding activity in rat liver, about three times that of fish liver. Elongation rates for the two species are nearly identical when body temperature difference is taken into account (from Q₁₀=3.0)¹⁸. An apparent excess of binding component (EFTu) also has been reported for *E. coli*¹⁹.

In contrast to the results for soluble EF1, direct assay for ribosome-bound EF1 in polyribosome preparations revealed significantly lower activity from thyroidectomised rats compared with controls (Fig. 1a). Salt-wash fractions obtained by extraction of polyribosome pellets with 0.5 M NH₄Cl also showed an approximately twofold difference in EF1 activity (Fig. 1b). Results are expressed relative to absorbance units of polyribosomal RNA from which the EF1 activity was derived. No other differences between polyribosome preparations from control and thyroidectomised rats were noted. Polyribosome recovery in the 240,000g pellet was the same for the two groups (2.9±0.4 mg RNA per g liver), as was the protein yield in the NH₄Cl extract (0.32±0.10 mg protein per mg RNA). Polyribosome profiles from liver post-nuclear supernatants of a matched pair of animals were indistinguishable from those of unoperated controls, as presented elsewhere²¹. Levels of EF2 activity in the NH₄Cl extracts (Table 2) were identical for normal and thyroidectomised rats.

Table 2 summarises the results for ribosome-associated EF1 and EF2. An EF1-dependent polymerisation assay

was also performed on the salt-wash fractions, with results comparable to those of the binding assay. The data show a 60% reduction in salt-extracted ribosome-bound EF1 activity in the thyroidectomised animals; the range at 95% confidence is 40%–75%. The observed change in elongation rate *in vivo*^{2,4} falls within this range.

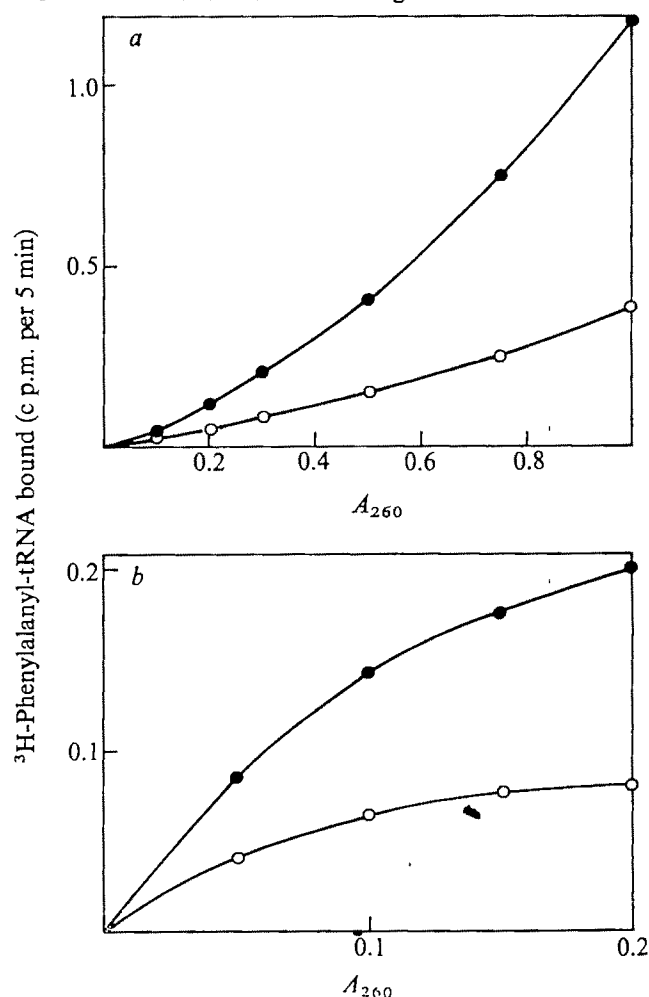


Fig. 1 a, EF1-dependent binding activity of polyribosome preparations of normal (●) and thyroidectomised (○) rat liver as a function of absorbance units at 260 nm of rRNA added to assay. b, EF1-dependent binding activity of supernatant fraction obtained by salt washing of polyribosomes as in (a), as a function of A₂₆₀ units of rRNA used in preparation. The salt-wash fraction was the supernatant layer (4 ml) recovered after the polyribosome suspension of 4 ml treated with 0.5 M NH₄Cl was centrifuged 1.5 h at 340,000g through a 9-ml layer containing 1.5 M sucrose, 0.5 M NH₄Cl and the other components of Medium A. The binding assay contained all the ingredients indicated in the legend of Table 1 with the exception of S-100 protein. Puromycin-treated ribosomes were also omitted in the direct assay of the polyribosome fraction

Table 2 Elongation factor activity in polyribosomes

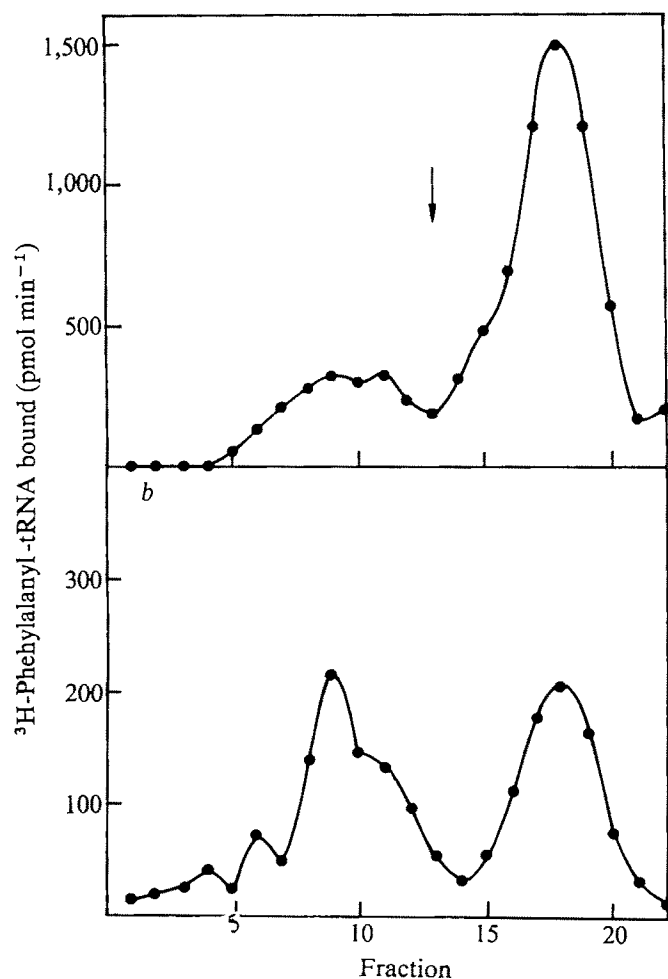
Group	Average body weight (g)	EF1 Binding Activity* Polyribosomes (Units per mg rRNA)	Salt-wash fraction (Units per mg rRNA)	EF2 Activity† Salt-wash fraction (Units per mg rRNA)
Normal	186	8.0±2.0 (14)	55±14 (12)	40±8 (12)
Thyroidectomised	133	3.2±1.0 (15)	24±8 (13)	45±6 (13)

*Assay as described in Table 1 with the exception of S-100 protein. Polyribosomes were assayed in the range of 0.1-0.5 A_{260} unit in the final volume of 0.12 ml; 30% of the apparent binding activity was found to be associated with polymerised species. Salt-wash fractions were assayed in the range of 0.01-0.05 A_{260} unit of rRNA used in each preparation; these fractions gave less than 5% of the reaction product in polyphenylalanine. Data are presented as mean±s.d. (number of animals). The preparation from each animal was assayed 3-5 times.

†EF2 was assayed in a polymerisation system as described in Table 1. In place of S-100 protein the system contained a saturating level ($5 \mu\text{g ml}^{-1}$) of purified rat liver EF1 and salt-wash fractions in the range of 0.02-0.1 A_{260} unit of rRNA used in preparation.

The molecular weight distribution of soluble and ribosome-bound EF1 activity from liver of normal and thyroidectomised rats was examined by sedimentation velocity in a sucrose gradient. As shown in Fig. 2a for a thyroidectomised rat preparation, EF1 activity in liver

Fig. 2 Sucrose density gradient analysis of soluble and ribosome-bound liver elongation factor 1. a, EF1 binding activity in post-ribosomal supernatant (S-100). b, EF1 binding activity in the salt-wash fraction obtained from purified polyribosomes. Both preparations shown were obtained from a thyroidectomised rat. The S-100 was diluted 1:2.5 with 0.05 M Tris-HCl, pH 7.5, 2 mM 2-mercaptoethanol, and 50 μl was layered on a 5.2 ml 5-20% sucrose gradient containing the same buffer. The salt-wash supernatant fraction was chromatographed on Sephadex G25 equilibrated with the same buffer, and 200 μl was layered on the gradient. Centrifugation was carried out for 4 h at 62,500 r.p.m. in a Spinco SW65 rotor. Bovine serum albumin, human γ -globulin and catalase were run as markers; the position of catalase (11S) is indicated by an arrow in (a). Aliquot portions of 20 μl for (a) or 50 μl for (b) were assayed in the binding system described in Table 1. Sedimentation coefficients relative to marker positions were calculated by use of tables³⁰ and converted to molecular weights by an approximate equation $M = 6.6 \times 10^3 S^{2/3}$ based on the standard proteins.



S-100 occurs predominantly in small forms peaking at about 80,000 daltons with a leading edge extending to about 200,000 daltons. The distribution is consistent with the forms of EF1 previously identified in rat^{22,23} and pig liver¹². In contrast, EF1 activity obtained by NH_4Cl extraction of polyribosomes is about equally divided between small forms and aggregates in the region of 350,000-600,000 daltons (Fig. 2b). Control rats gave results identical to those of thyroidectomised rats.

The results above indicate that a component subject to assay in the polyribosome fraction may be the controlling factor in aminoacyl-tRNA binding and in polypeptide chain elongation in relation to thyroid hormone. It is apparently different from the major soluble binding activity, which does not change with hormonal status (Table 1). Some support for a qualitative difference in EF1 activity in the two compartments is provided by the sucrose gradient profiles (Fig. 2). Differences between soluble and ribosome-bound activity have also been reported for wheat embryo EF-1 (refs 24 and 25). The rate-limiting component could be a recycling factor, analogous to the bacterial elongation factor EFTs (ref. 26). Evidence for recycling of the activity associated with the binding component of pig liver EF-1 has been reported recently^{27,28}.

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Sodium ions and the shut-off of host cell protein synthesis by picornaviruses

TRANSLATIONAL control mechanisms have an important role in many cellular processes^{1,2}, but there is as yet little evidence showing the mechanism by which the control of protein synthesis at the translational level occurs. One of the best illustrated examples of translational control is observed when picornaviruses infect susceptible animal cells³. There is a rapid inhibition of cellular protein synthesis (the shut-off phenomenon) followed by a burst of viral protein synthesis, during which virtually no cellular proteins are made^{4,5}. Cellular mRNAs are not degraded with infection^{6,7}, but in spite of their presence the cellular protein-synthesising machinery only recognises and translates viral mRNAs.

In trying to formulate a model to explain the shut-off phenomenon the following experimental observations have to be accommodated (1) the inhibition of host protein synthesis occurs at the initiation step⁸. (2) No replication of the virus is necessary for the shut-off to occur; under these conditions the shut-off depends upon the multiplicity of infection⁴ (3) A viral protein factor is required; mutants defective in the ability to shut off map in a region of the genome that codes for the structural proteins⁹ (4) Cell-free protein synthesising systems obtained from uninfected cells

or cells infected with picornavirus are equally active in translating viral or host mRNAs *in vitro*^{10,11}. Together these data rule out the possibility of a specific modification of the protein-synthesising machinery after viral infection or the appearance of a stable macromolecular inhibitor such as double-stranded RNA^{12,13}, or a viral protein¹⁴. On the other hand the shut-off mechanism cannot be explained only by a direct competition between viral and cellular mRNAs¹⁵, because this is inconsistent with the observations mentioned above. Moreover, if cellular mRNAs were simply replaced by viral mRNAs this would not lead to the inhibition of total protein synthesis which is always found in infected cells.

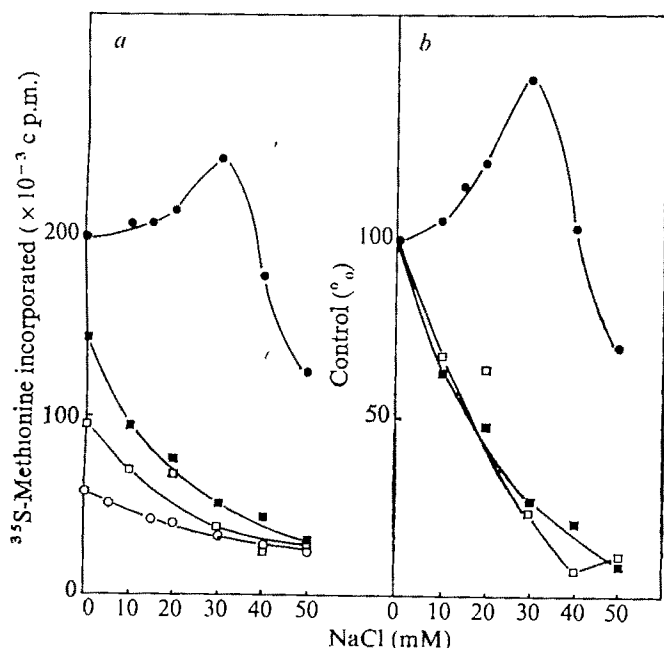
Increasing evidence has accumulated to show that after viral infection there are changes in the structure of cellular membranes^{16,17}. One likely consequence of this is a disruption of the ionic gradient between the inside and the outside of the cell. Indeed it has been shown that when *Escherichia coli* is infected by T7 bacteriophage, the membrane becomes leaky¹⁸, leading to a change of potassium concentration inside the cell¹⁹. The importance of such changes in monovalent cation concentration for cellular processes was pointed out by Lubin, when he observed that the rate of macromolecular synthesis depends absolutely upon the potassium concentration inside the cell²⁰. More recent experiments, notably those of Koch and his colleagues, also indicate the importance of monovalent ions in cellular processes; for example hypertonic medium causes the run off of HeLa cell polysomes²¹. Interestingly, if the cells are infected with poliovirus, viral protein is resistant to this inhibition²².

We here propose a model for shut-off which we believe takes into account all the observations mentioned. We suggest that following infection by picornaviruses there are changes in the cellular membrane which lead to an influx of sodium ions, and that this change in monovalent cation concentration results in the differential translation of host and viral mRNAs and leads to the shut-off of cellular protein synthesis. To test this we first examined the translation of different mRNAs *in vitro* in different salt conditions. Figure 1 shows that sodium ions cause an inhibition of protein synthesis directed by globin mRNA or by total mouse cell poly(A)-containing RNAs, in a cell-free system from ascites cells containing 80 mM KCl. A concentration of 30 mM NaCl inhibits protein synthesis directed by these mRNAs by about 80%. In the same cell-free conditions however, this concentration of sodium actually stimulates synthesis of the products directed by EMC RNA by almost 50%. A similar result was obtained with RNA from two other picornaviruses, mengovirus and poliovirus (unpublished observations). Polyacrylamide gel analysis of the proteins synthesised in the presence of sodium ions (Fig. 2) revealed an increase in the amount of the EMC-specific polypeptides and an inhibition of the synthesis of cellular proteins. Furthermore, the changes seen are only in the amount of protein synthesised, not its molecular weight.

The *in vitro* discrimination by sodium between viral and cellular protein synthesis occurs in various conditions. Figure 3 shows that the differences exist at all times during the incubation period and using different mRNA concentrations. In the control incubations containing no added sodium ions, approximately equal amounts of ³⁵S-methionine are incorporated in response to EMC RNA or cellular poly(A)-containing mRNAs, whereas, on addition of NaCl the translation of viral mRNA is favoured.

EMC RNA is efficiently translated *in vitro* over a wide range of potassium ion concentrations with a maximum at 110 mM KCl, whereas the translation of cellular mRNAs has a more stringent potassium ion requirement with a sharper optimum at about 80 mM KCl (Fig. 4). Addition of NaCl to the cell-free system shifts these optima such that in the presence of 30 mM NaCl maximal EMC RNA trans-

Fig. 1 Effect of sodium chloride on cellular and viral protein synthesis. Reaction mixtures contained (in 25 μ l): 20 mM HEPES, pH 7.0, 80 mM KCl, 1.5 mM MgCl₂, 6 mM 2-mercaptoethanol, 50 μ M spermine, 250 μ M spermidine, 200 μ M of each of the unlabelled L-amino acids minus methionine, 10–16 μ Ci of ³⁵S-methionine (specific activity 250–400 Ci mmol⁻¹), 1 mM ATP, 0.2 mM GTP, 10 μ l ascites S30, and where indicated 1 μ g EMC RNA, 1 μ g globin mRNA or 1 μ g mouse cell total poly(A)-containing mRNAs. The reaction was incubated for 90 min at 34 °C. At the end of this period 2 μ l of the reaction were withdrawn to analyse protein synthesis by trichloroacetic acid precipitation and subsequent filtration on nitrocellulose filters. The rest of the reaction was used to analyse the proteins on polyacrylamide gels as described²³. \circ , Endogenous protein synthesis; \bullet , plus EMC RNA; \square , plus globin mRNA; \blacksquare , plus mouse cell poly(A) containing RNA. *a*, Incorporation of ³⁵S-methionine at different sodium chloride concentrations; *b*, results expressed as a percentage of incorporation in the incubation containing no added sodium chloride.



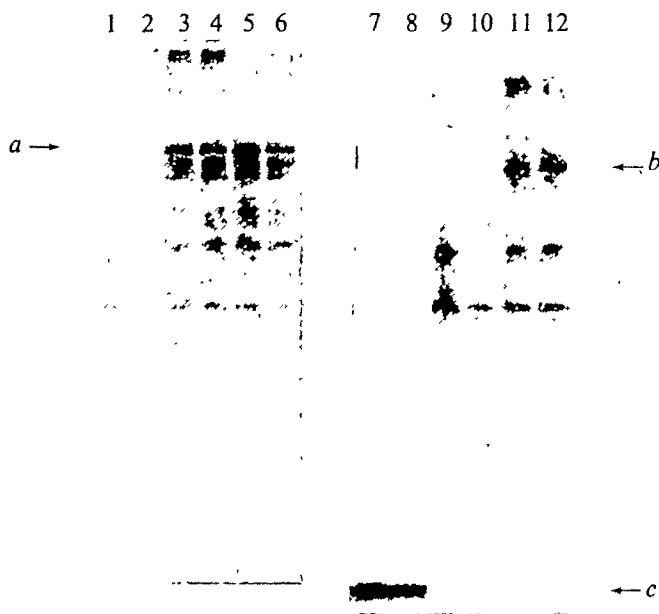


Fig. 2 Autoradiography of a polyacrylamide gel of the proteins synthesised *in vitro* under different conditions. 1, Endogenous protein synthesis under normal conditions (similar to those described in Fig. 1); 2, plus 30 mM NaCl; 3, plus 1 μ g EMC RNA; 4, 1 μ g EMC RNA, plus 15 mM NaCl; 5, 1 μ g EMC RNA, plus 30 mM NaCl; 6, 1 μ g EMC RNA, plus 45 mM NaCl; 7, 1 μ g globin mRNA; 8, 1 μ g globin mRNA + 30 mM NaCl; 9, 1 μ g cellular poly(A)-containing mRNAs; 10, 1 μ g cellular mRNAs plus 30 mM NaCl; 11, 2 μ g poliovirus RNA; 12, 2 μ g poliovirus RNA, plus 30 mM NaCl. A, B and C indicate the major products synthesised in response to EMC RNA, polio RNA and globin mRNAs respectively.

lation occurs at about 90 mM KCl. The KCl optimum for cellular mRNA also shifts on addition of NaCl, and using the conditions just mentioned (that is, 90 mM KCl + 30 mM NaCl), virtually no cellular protein is synthesised. Thus we conclude that it is the higher monovalent ion concentration in the incubation mixture which discriminates between the translation of EMC RNA and cellular mRNAs and not necessarily the presence of sodium ions. If under conditions of shut-off *in vivo* there were an ion influx, however, the physiological ion involved would be sodium.

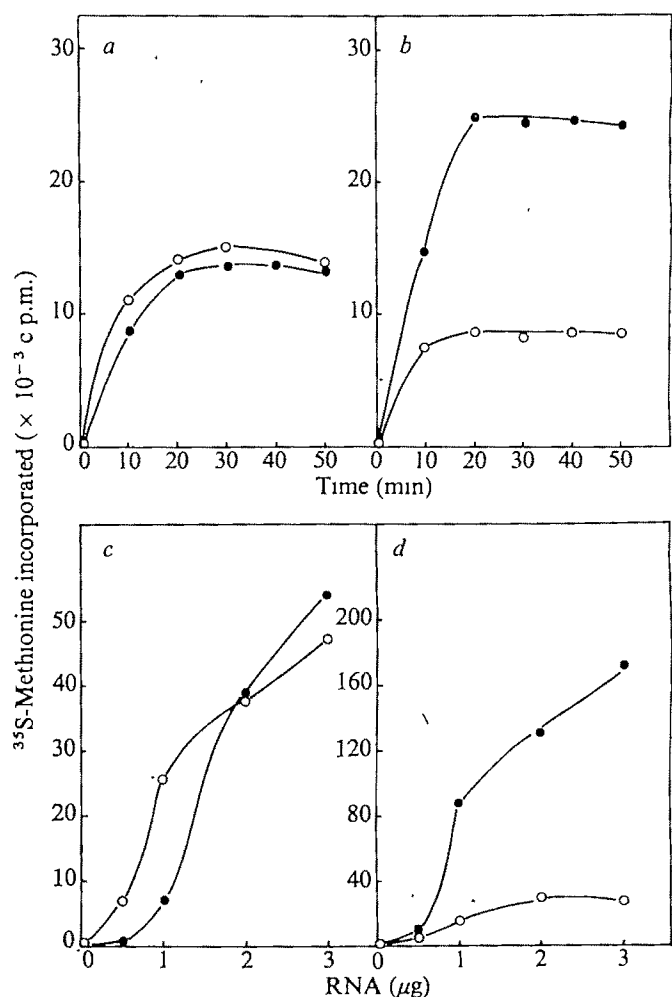
Earlier experiments have shown that the elongation rate *in vitro* is increased at higher potassium concentrations and consequently the product made in these conditions is of higher molecular weight²², this raises the possibility that the stimulation by sodium shown here is merely a nonspecific effect on the elongation phase of protein synthesis *in vitro* which gives a differential response because EMC RNA is much longer than an average cellular mRNA. This is not the explanation in this case, however, because, as shown in Fig. 2, in our conditions the effect of higher salt concentration is quantitative and results in the synthesis of greater amounts of the protein coded for by the viral RNA but does not change its molecular weight. Thus this result is consistent with the view that there is more initiation on EMC RNA when sodium is present rather than more efficient elongation.

To examine the problem directly we have analysed the effect of sodium on the initiation of protein synthesis directed by globin mRNA or EMC RNA using the sparsomycin technique³. Addition of sodium stimulated the formation of the EMC di- and tripeptides by up to 100% whereas the dipeptide formed with globin mRNA was decreased by about 60% (data not shown). We conclude that sodium inhibits the initiation of protein synthesis directed by globin mRNA and stimulates initiation directed by EMC RNA. At present we do not know what step in initiation is affected by sodium, but we believe that it probably has a

direct effect on the interaction between mRNA and ribosomes, thereby increasing the affinity of picornavirus RNA and decreasing the affinity of cellular mRNAs.

The experiments outlined above all indicate that sodium ions can discriminate between the translation of host and picornavirus mRNAs *in vitro*, and are consistent with our model for shut-off. A further prediction of our model is that, following infection, the intracellular concentration of sodium ions increases. Indirect evidence for this was obtained by measuring the activity of the enzyme responsible for the maintenance of the monovalent ion gradient (the Na^+/K^+ ATPase)²⁴ at different times after infection by estimating the uptake into cells of the potassium ion analogue $^{86}\text{Rb}^+$. In parallel experiments, viral protein synthesis was first observed 4 h post-infection and maximum synthesis was reached at 5–6 h post-infection (data not shown). Concomitant with the onset of viral protein synthesis the ability of the cell to take up $^{86}\text{Rb}^+$ becomes drastically impaired (Fig. 5). This indicates that either the Na^+/K^+ ATPase activity is severely inhibited or that the plasma membrane becomes leaky to monovalent ions. Either mechanism will result in the breakdown of the monovalent ion gradient, and lead to an increase in the sodium ion concentration inside the infected cell. Such evidence supports our hypo-

Fig. 3 Time course and RNA dependence of protein synthesis directed by EMC RNA or cellular poly(A)-containing mRNAs. Reaction mixture contained in 50 μ l the same components as indicated in Fig. 1 except that the specific activity of ^{35}S -methionine was 20 Ci mmol⁻¹. The potassium concentration was 80 mM. Endogenous protein synthesis was subtracted from each of the values given. ●, EMC RNA directed protein synthesis; ○, protein synthesis in response to total mouse cell poly(A)-containing mRNAs. a, c, Controls; b, d, + 30 mM NaCl



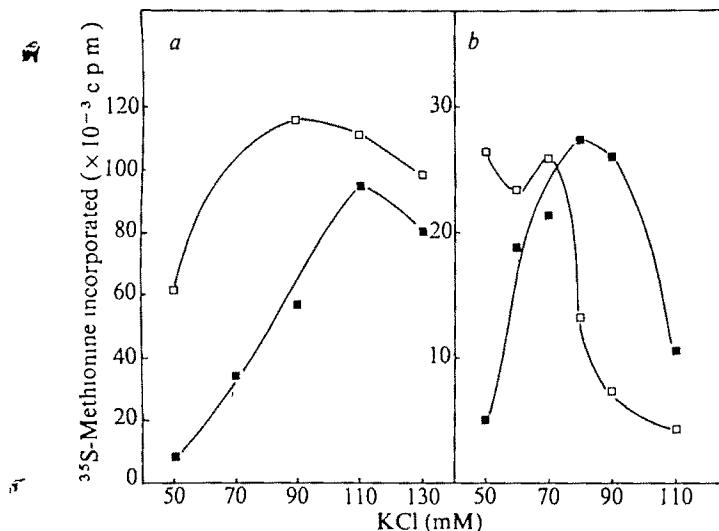


Fig. 4 Effect of potassium chloride concentration on protein synthesis in response to EMC RNA and cellular mRNAs in the presence and absence of sodium chloride. Conditions and components were basically as indicated in Fig. 2. *a*, Protein synthesis in response to EMC RNA and *b*, in response to total cellular poly(A) mRNAs. ■, No added sodium; □, plus 30 mM NaCl. Endogenous incorporation was subtracted.

thesis that a redistribution of ions occurs following viral infection.

To summarise, we suggest that the shut-off phenomenon could be explained by supposing that after infection a viral protein is able to alter the gradient of monovalent ions maintained by the cell membrane, increasing the concentration of sodium in the cytoplasm and causing inhibition of

the initiation of cellular protein synthesis (polysomal run-off). In addition, the new ionic conditions in the cytoplasm would facilitate the translation of viral RNA because (1) there would be no competition from host mRNA and (2) the binding of viral RNA would be favoured by the increased monovalent ion concentration. Experiments examining the effects of sodium ions on the cell-free translation of host and viral mRNAs, and indirect measurements of the ionic gradient in virus-infected cells support the model.

The model presented here raises many questions. How does a viral protein change the ionic environment within the cell? Is this absolutely necessary for viral development? Does the change in ionic environment affect other cellular functions? Do all viruses use a similar strategy? It is equally possible that by the appropriate regulation of ion pumps, protein synthesis in uninfected cells could also be regulated by a similar mechanism since it is already known that cellular mRNAs also display a spectrum of resistance to excess salt.

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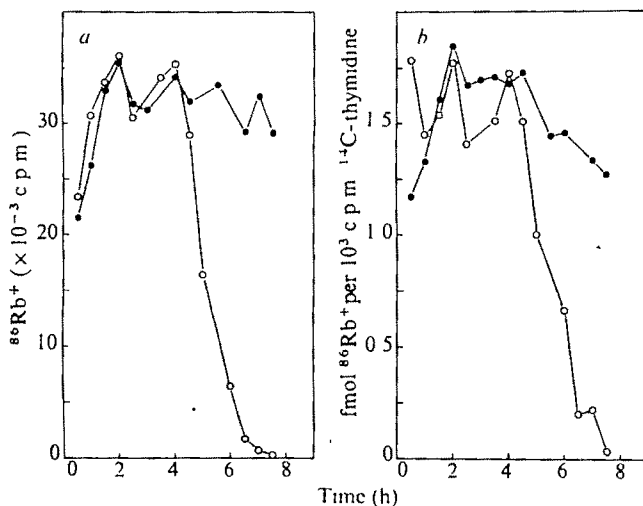
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Fig. 5 Mouse fibroblast 3T6 cells were grown in 3 cm diameter dishes containing 2 ml E4 medium with 10% calf serum and ^{14}C -thymidine (50 mCi mmol $^{-1}$, 0.05 $\mu\text{Ci ml}^{-1}$). When the cells were confluent, the medium was removed and the cells infected with encephalomyocarditis virus (20 PFU per cell). After 1 h at 37°C, 1 ml E4 medium containing 5% calf serum was added. The final concentration of KCl in the medium was 0.5 mM. Control or virus-infected cells were pulse labelled for 30 min at different times after infection with 1.25 $\mu\text{Ci ml}^{-1}$ $^{86}\text{Rb}^{+}$ (370 Ci mmol $^{-1}$). The medium was removed and the cell monolayer washed three times with phosphate buffer. 1.5 ml 5% trichloroacetic acid was added to fix the cells and the $^{86}\text{Rb}^{+}$, which leaks out from the cells, estimated by taking a 1 ml sample of the supernatant and measuring the Cerenkov radiation in a scintillation counter. The cell monolayer was washed several times with 5% TCA and twice with methanol. 0.2 ml of 0.02 N NaOH was added and a 0.150 ml sample was taken to estimate the ^{14}C -thymidine incorporated. *a*, Total c.p.m. of $^{86}\text{Rb}^{+}$ taken into the cells. *b*, Results of the $^{86}\text{Rb}^{+}$ uptake normalised to the ^{14}C -thymidine incorporated (●) $^{86}\text{Rb}^{+}$ uptake in control cells (○), $^{86}\text{Rb}^{+}$ uptake in encephalomyocarditis virus-infected cells.



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Purification and characterisation of viral RNA of a sarcoma virus isolated from a woolly monkey

RNA-CONTAINING type-C viruses with the ability to induce morphological transformation of fibroblasts in cell culture have been isolated from both avian and mammalian species. In studies on such viruses, clonal isolates of the Rous sarcoma virus have been identified which are competent both for replication and for transformation¹. In contrast, in mammalian systems virtually all strains of sarcoma viruses thus far isolated are defective by themselves for replication². In order to obtain infectious sarcoma virus, helper type-C viruses must also be present to provide

functions necessary for replication. To analyse the RNA subunits contained in heterologous type-C virus pseudotypes of defective sarcoma viruses, we have studied the RNA components obtained from a virus complex containing the woolly sarcoma virus and a mouse type-C helper virus. We have observed that the 45 to 70S high molecular weight RNA formed in this virus complex represents two distinct populations of high molecular weight RNA. One population is composed of subunits of mouse viral RNA, and the other population is composed of subunits of woolly sarcoma virus RNA. These two sets of apparent dimers²⁻³ have been readily separated from each other in sucrose gradients, thus providing a rapid, simple approach to obtaining large quantities of sarcoma virus RNA free of helper virus RNA. Using this approach, the nucleic acid sequences of the woolly sarcoma virus have been analysed.

In past experiments, the sarcoma virus first isolated from a spontaneous fibrosarcoma of a woolly monkey^{4,7}, was subsequently cloned in transformed nonproducer rat cells free of replicating woolly leukaemia virus^{8,9}. By infecting the non-producer cell⁸ with an amphotropic strain of mouse type-C virus¹⁰ we have produced a pseudotype containing the amphotropic mouse type-C helper virus and the woolly sarcoma virus. This complex was transmitted to BALB/c mouse cells and foci producing both viruses were cloned. One such clone of transformed cells, WB334, was analysed by RNA-³H-DNA hybridisation techniques for the relative viral RNA content of the mouse helper virus and the woolly sarcoma virus. To detect the mouse type-C viral RNA, a cDNA probe was prepared from the amphotropic virus grown in a strain of wild mouse cells, SC-1 (ref. 10). To detect the RNA content of the woolly sarcoma virus subunit, a cDNA probe from the woolly leukaemia virus was used since earlier experiments, hybridising Wo-1v ³H-cDNA to RNA of non-producer cells transformed by the Wo-sv, have indicated that the woolly sarcoma viral genome contained sequences homologous to the woolly leukaemia virus¹¹. A virus preparation from the WB334 clone was prepared and the RNA isolated in sucrose gradients as shown in Fig. 1.

Each fraction of the sucrose gradient was hybridised to the cDNA from the mouse type-C virus and to the cDNA from the woolly leukaemia virus. Two discrete peaks of hybridisation can be seen; under these stringent hybridisation conditions the faster sedimenting peak hybridises well to the cDNA homologous to the mouse type-C virus and the slower sedimenting peak hybridises to the cDNA from the woolly leukaemia virus. The peak tube of each of the two RNA peaks is separated by approximately five fractions in the sucrose gradient. In addition, there is no indication of high molecular weight RNA heteromers composed partly of mouse and partly of woolly RNA since the hybridisation profiles are almost completely non-overlapping. Thus, the results indicate that this pseudotype mixture contains two distinct populations of high molecular weight RNA. Assuming that the two-subunit model for the high molecular weight RNA of the tumour viral genome is correct²⁻³, then the faster sedimenting peak represents homodimers of amphotropic viral RNA and the slower sedimenting peak homodimers of woolly viral RNA. In experiments not shown, the high molecular weight form of each RNA was heated for 2 min at 80 °C and resedimented in sucrose gradients. After denaturation, the mouse viral RNA sedimented at 33–35S and the woolly sarcoma viral RNA at 24–26S.

In Fig. 1b, a large quantity of the virus mixture was disrupted with sodium dodecyl sulphate, and the RNA sedimented in sucrose gradients. The optical density at 260 nm was determined in each fraction. Two discrete peaks of absorbance can be seen similar to the results

obtained above in the hybridisation experiment shown in Fig. 1a.

The RNA in the slower sedimenting high molecular weight RNA was isolated from the sucrose gradient shown in Fig. 1b, and reverse transcribed *in vitro* with partially purified avian myeloblastosis virus reverse transcriptase¹¹. The ³H-cDNA probe prepared from the woolly sarcoma viral RNA was tested for its information content and the results were compared with a cDNA probe prepared from

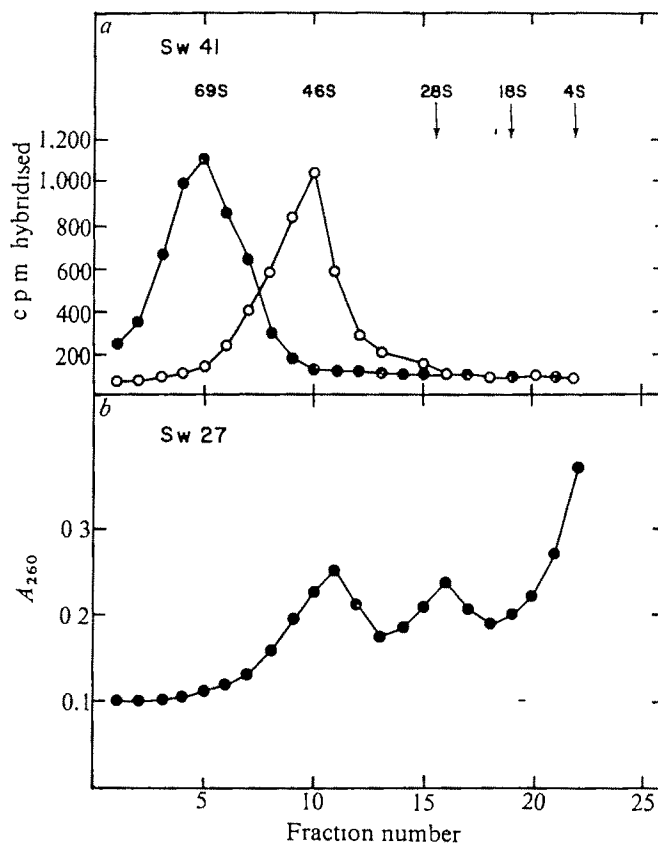


Fig. 1 Sucrose gradient analysis of viral RNA. *a*, Analytical gradient: 60 ml of supernatant fluid, representing a 6-h harvest of the culture fluid from the WB334 culture, were clarified at 5,000 r.p.m. at 4 °C for 10 min in a PR6000 centrifuge. The fluid was applied in 20 ml aliquots to a discontinuous sucrose gradient in an SW27 rotor tube containing: 4 ml of 60% sucrose and 12 ml of 30% sucrose prepared in 0.02 M Tris-HCl pH 7.5, 0.1 M NaCl, 0.001 M EDTA (TNE). The virus was centrifuged on to the 60% cushion at 25,000 r.p.m. at 4 °C for approximately 16 h. The virus was aspirated from the 60% cushion, diluted four-fold with TNE and centrifuged at 100,000g at 4 °C for 2 h to concentrate the virus. The pellet was resuspended in 1.0 ml containing TNE and 1% sodium dodecyl sulphate. The disrupted virus was then centrifuged in a 15–30% sucrose gradient in TNE in an SW41 rotor tube at 18 °C for 3.25 h at 39,000 r.p.m. 25 fractions of approximately 0.5 ml were collected by puncturing the tubes from below. 20 µg of yeast RNA were added to each fraction and the fractions precipitated with 2 volumes of ethanol. After collecting the ethanol precipitates, the fractions were resuspended in 100 µl of 0.02 M Tris-HCl pH 7.2 and 10 µl of each fraction was assayed for hybridisation as detailed below. Each hybridisation reaction contained ³H-cDNA (1 × 10⁷ c.p.m. µg⁻¹) either from an amphotropic mouse leukaemia virus (2,100 c.p.m.) or from the woolly leukaemia virus (2,500 c.p.m.). The amphotropic virus was grown in SC-1 cells, and the woolly virus in NC37 human cells¹¹. Hybridisation was performed and analysed with the use of sl nuclease by procedures fully described in earlier publications¹¹. *b*, Preparative gradient: 3 ml of the virus mixture produced from 25 l of the pseudotype virus mixture was disrupted with 1% sodium dodecyl sulphate and centrifuged for 4.5 h at 24,000 r.p.m. in an SW27 15–30% sucrose gradient at 18 °C. Fractions of 1.0 ml were collected by puncturing tubes from below, and the absorbance of each fraction at 260 nm was recorded. For the subsequent studies of the woolly sarcoma virus RNA, fractions 14 to 18 in Fig. 1b were pooled, and the RNA collected by precipitation with ethanol and centrifugation of the ethanol precipitate.

woolly leukaemia viral RNA. As shown in Table 1, the cDNA prepared from the woolly leukaemia virus RNA hybridises to both the woolly leukaemia virus and woolly sarcoma viral RNA; at saturation (C_{rt} of 1.0 mol l^{-1}) approximately 50% of the sequences contained in the woolly leukaemia cDNA are protected by the woolly sarcoma virus RNA. The results indicate, as previously suggested, that the woolly sarcoma virus contains only a portion of the woolly leukaemia genome¹¹.

The crude cDNA probe prepared from the woolly sarcoma viral RNA, which contains mostly woolly leukaemia virus sequences, was also hybridised to the same viral RNAs and showed slightly greater hybridisation to the woolly sarcoma virus RNA than to the woolly leukaemia virus RNA. After purification of the woolly sarcoma virus probe to remove woolly leukaemia virus sequences (see legend to Table 1), the woolly 'src' probe was re-tested for its specificity and the results are also shown in Table 1. The woolly src probe hybridised well to the woolly sarcoma virus RNA and not at all to the viral RNA from the woolly leukaemia virus, or viral RNA from RD114 virus, RT21c rat virus, or amphotropic mouse leukaemia virus. In addition, the woolly viral src sequences were detected in cellular RNA in non-producer rat cells transformed by the woolly sarcoma virus but not in rat cells transformed by the Schmidt-Ruppin strain of Rous sarcoma virus. These src sequences were also detected in horse cells producing both the woolly leukaemia and woolly sarcoma viruses but not in horse cells producing the woolly leukaemia virus alone. The results show that woolly sarcoma virus contains two sets of nucleic acid sequences. One set of the sequences is contained in the woolly leukaemia virus, and another set is specific for the woolly sarcoma virus. These results are consistent with finding on other avian and mammalian sarcoma viruses which showed their genomes to contain both leukaemia virus sequences and distinctive sequences specific to the sarcoma viruses^{2,12,13}. In order to quantitate the relative percentage of the two sets of sequences in the woolly sarcoma virus genome, ³²P radio-labelled RNA was prepared from the woolly sarcoma virus, by labelling the viruses

released from WB334 cells with inorganic ³²P and separating the high molecular weight RNAs as shown in Fig. 1b. The ³²P-labelled woolly sarcoma virus RNA was then hybridised to a two-fold molar excess of ³H-cDNA from the woolly leukaemia virus, or to a two-fold excess of cDNA purified to represent the novel sequences in the woolly sarcoma virus. At these ratios of DNA to RNA, the src probe protected 52% of the ³²P-labelled RNA and the Wo-lv cDNA protected 52% of the ³²P-labelled RNA.

Thus we have analysed the RNA subunits contained in the virus complex containing an amphotropic mouse type-C virus pseudotype of the woolly virus. The data indicate that the high molecular weight-forms of the two viral RNAs in this virus complex are essentially completely separable by velocity sedimentation in sucrose gradients and that no detectable heterodimers occur between the mouse type-C viral RNA and the woolly sarcoma viral RNA. The woolly sarcoma viral RNA is smaller than the accompanying helper viral RNA, and contains only a portion of the Wo-lv genome. In the woolly sarcoma viral RNA a set of sequences has been detected which comprise approximately 15% of the woolly sarcoma genome; the sarcoma virus specific sequences are not detected in the woolly leukaemia virus. Thus, the genome of the naturally occurring Wo-sv seems to be similar to the genomes of rodent sarcoma viruses formed in laboratory experiments^{14,15}; that is the sarcoma virus genome represents a set of new sequences plus a portion of the genome of the helper virus contained in the original virus stock. The ability to obtain large quantities of purified RNA and cDNA specific for the woolly sarcoma virus should allow an identification both of the origin of the sarcoma-specific sequences of the woolly sarcoma virus and possibly by *in vitro* translation, identification of any possible gene product(s) of these sequences.

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Table 1 Hybridisation with woolly leukaemia and woolly sarcoma virus cDNAs

RNA Source	c.p.m. hybridised with ³ H-cDNA from		
	Wo-lv	Crude Wo-sv	Purified Wo-sv
Viral			
Wo-lv	2,116	965	18
Wo-sv	1,158	1,150	33
RD-114	41	98	10
Amphitrope	186	156	25
RT21C	38	108	13
None	43	93	10
Cellular			
Non-producer cells			
Wo-sv NRK	—	—	333
SR NRK	—	—	22
Producer cells			
Wo-lv NRK	2,230	—	12
Wo-lv horse	2,165	—	12
Wo-lv/Wo-sv horse	2,177	—	308

Each hybridisation with woolly leukaemia virus ³H-cDNA contained 2,500 TCA c.p.m. The hybridisations with the crude woolly sarcoma virus cDNA contained 1,800 TCA c.p.m. and with the purified woolly sarcoma virus cDNA 630 TCA c.p.m. The sarcoma-specific cDNA was obtained by hybridising the crude cDNA to woolly leukaemia virus RNA and subsequent chromatography on hydroxylapatite^{14,15}. The hybridisations with viral RNA were carried out to C_{rt} values of 1.0 mol l^{-1} and with cellular RNA to C_{rt} values of $5 \times 10^3 \text{ mol l}^{-1}$. Analytical hybridisation conditions and analysis with the use of sl nuclease have also been fully detailed in earlier publications¹¹. The source of the cells has been described¹⁴ except for the horse cell which was obtained from the American Type Cell Collection.

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Errata

In the letter "Stab initiation of explosions" by M. M. Chaudhuri (*Nature*, **263**, 121; 1976) the expression giving q_1 in equation (2) should be

$$q_1 = \left[1 + \sqrt{\left(\frac{\pi}{2} \frac{v(1 - \sin^2 15^\circ) R p C}{4\lambda} \right)} \right]^{-1}$$

matters arising

Fossil hominid femora

McHENRY and Corruccini used a canonical variates analysis to assess the affinities of five early hominid femora¹, two of which are complete (ER 1472, 1481, although the head of the former is damaged). The fossil hominids appeared to separate from living *Homo* on the fourth variate axis, accounting for only 4.7% of the variation. Nonetheless, the authors conclude that "there were at least two distinctive forms of hominids" represented. The authors state that head size is "heavily weighed" on this axis and suggest "large femoral heads might be the result of a widened birth canal." Yet, differences in femoral head size must be related to bone length to be meaningful and unambiguous; larger specimens will probably have larger heads.

Have they truly determined a relative head size variable? They argue that the femoral dimensions are adjusted using average within-taxon allometry coefficients "between each measurement and a standard size variable". But which coefficients might be used to standardise the early hominid measurements is problematic, for these combine relative neck length and shaft thickness dimensions which are unknown for any other hominid taxon, except possibly *H. erectus*.

Because they divide head diameter by neck length or shaft thickness, there is confusion about whether the head is relatively small, or the neck and shaft are relatively large. This underlies the different estimates of bone length in fragmentary early hominid specimens. For instance, McHenry² rejected an estimate of early hominid femoral length based on a regression from head size³ because, he argued, the heads are relatively small. Yet, he used a multiple regression to predict bone length giving much longer determinations based in part on neck length³, although later realising that the necks are relatively long⁴. Thus, the necessity of relying on those few specimens with known or reconstructable shaft lengths is underscored: shaft length is the only size measure which does not

require choice between neck or other shaft measures to estimate size in an analysis intended to determine whether or not the heads were relatively small. Unfortunately, only one published specimen requires no reconstruction to avoid this circularity (ER

Libben mean. The only other specimen that can be considered is STS 14, a very damaged proximal femur with a reconstructed length of 285 mm (ref. 5). (The estimate used here adds a portion of the head diameter inadvertently left off the published length of

Table 1 Biomechanical neck length and head diameter relative to femur length in a sample of 50 Libben Amerinds

	Relative biomechanical neck length	Relative head diameter
Mean \pm (s.d.)	0.154 (0.008)	0.100 (0.005)
Maximum	0.177	0.114
Minimum	0.135	0.091

These data were provided by C. O. Lovejoy.

1481), although three others make possible additional estimations.

When 50 males and females from the Libben Amerind collection (Table 1) are used for comparison, relative biomechanical⁴ neck length for ER 1481 is 3.25 standard deviations above the Libben mean, and relative head diameter 1.80 standard deviations above the mean (Table 2). The next best specimen is ER 1472. Length is known but damage to the head prevents accurate head or biomechanical neck length measurements. As preserved, relative neck length is too short, but is still 1.00 standard deviation above

276mm). The neck is broken just before the junction with the head and has been repaired, although not reconstructed with plaster. The head diameter is reconstructed, but constrained because it must fit the associated acetabulum. Relative neck length is 3.25 standard deviations above the Libben mean, and relative head size 1.80 standard deviations. No attempt has been made to reconstruct femur length in other specimens with heads, since the various shaft diameters show a very low correlation with length in specimens with known or reconstructable lengths.

Table 2 Relative biomechanical neck length and head diameter in early fossil hominids compared with Libben

	Relative biomechanical neck length		Relative head diameter	
	Calculated	Difference from Libben mean in s.d.	Calculated	Difference from Libben mean in s.d.
ER 1481	0.180	+3.25	0.109	+1.80
ER 1472	>0.162	>+1.00		
STS 14	0.182	+3.50	0.109	+1.80
ER 999	0.164	+1.25		

These measurements were taken by me.

Libben mean. ER 999, from above the Chari tuff, (1.34 Mys) is probably *H. erectus*; the relative neck length is 1.25 standard deviations above the

In sum, for all the known or reasonably reconstructable specimens, neck length is relatively very large compared with living humans and

Table 3 Comparison of innominate measurements normalised to acetabulum height

	Libben mean	σ	Distance from Libben mean in s.d.			
			STS 14	SK 3155	SK 50	OH 28
Ratio to acetabulum height of:						
Ilium height	2.33	0.14	+2.8	> +3.4*		
Greater sciatic to anterior notch	1.37	0.08	-2.3	-0.05	+0.46	-1.52
Anterior inferior to ischial spine	1.98	0.09	-1.3	-2.2		-2.9
Anterior inferior to posterior superior spine	2.57	0.14	-2.4	1.7		-0.4
Functional length of ischium	1.45	0.08	-3.8	-4.9	-0.8	-4.5

These measurements were taken by me. The functional length of the ischium⁴ is measured on both the Australopithecines and the Libben sample, the breaks on the STS 14 ischium and on the SK 3155 ischium minimise the measurement. The Libben sample size is 30. That some of these measures which cross, or almost cross the acetabulum differ considerably from the Libben mean when normalised by acetabulum size further emphasises the differences in proportion; when normalised to the acetabulum the iliac blades are relatively large and most other measures small, but normalised to the ilia everything else is extremely small.

*Addition of the unfused iliac crest would make this larger.

relative head size is, if anything, above the human mean⁴. These head, neck and shaft length relations are shown by two femora attributed to "*Homo*," and one attributed to "*Australopithecus*." Moreover, judging from the published photographs they also characterise the complete Afar hominid femur attributed to "*Australopithecus*"⁸. Former claims of relatively small head size come from comparison of head diameter with shaft diameter in incomplete specimens. Analysis of femora of known or reconstructable lengths shows that the shaft diameters were relatively large.

The demonstration that the heads were not relatively small calls to question the claim that the acetabula were relatively small¹, since of all pelvic dimensions the acetabulum has the highest correlation with femur length (0.840 for 30 Libben Amerinds). Schultz⁷ demonstrated the large relative size of the STS 14 and reconstructed SK 50 acetabula. When various comparable innominate measurements are normalised to acetabulum height (Table 3), and compared with the Libben sample, only relative ilium height is consistently above the Libben mean, as expected given the greater amount of iliac flare in the early hominids⁴. Iliac breadths are generally below the Libben mean, except for one measure of SK 3155, and contrary to "conventional knowledge", the functional length of the ischium is relatively short. The *H. erectus* innominate follows the australopithecine pattern. Only if normalised to iliac height would the early hominid acetabulum appear relatively small, and the other measurements would be relatively yet further below the Libben mean. Given the above, it is far more likely that australopithecine acetabula are human-like relative to body size, and the ilia are relatively long.

In sum, the distinguishing features of the early hominid hip complex reveal a pattern of form differing from living humans because of a combination of narrower birth canal and

markedly greater muscular activity, but not differing in locomotor capacity.

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McHENRY AND CORRUCINI REPLY—The discussion here¹ is about different fossils. We show that taken together the relative proportions of the proximal ends of KNM-ER 1481 and 1472 are distinguishable from those of SK 82 and 97 and KNM-ER 1504. Wolpoff argues¹ that the reconstructed femoral head diameter and neck length relative to reconstructed femoral length in STS 14 are similar to KNM-ER 1481. We feel that a meaningful and unambiguous argument should not be based upon measurements of a fossil (STS 14 femur) described as "a useless jumble of glued fragments surmounted by a crude plaster head and neck"². Nor should the length of a half missing ischium (SK 3155)³⁻⁶ be used to bolster such an argument.

A few points need to be clarified. (1) Our analysis is based on both univariate and canonical variates analysis and the results agree. (2) The variation on the fourth canonical variate is not interpreted by us as the result of femoral head size only. In fact, greater trochanter projection has the highest correlation with that variate, followed by neck length and femoral head size. The canonical variate is complex, reflecting the complexity of interrelationships among the variables. (3) Because variance scales to the number of subjects, one would not expect a high percentage of

variance on the fourth canonical variate. The fourth variate contributes substantially to the multivariate distances between subjects. (4) KNM-ER 1481 does indeed have a relatively big head, which is just our point because it represents the *Homo* taxon. The ratio of femoral head size to length in KNM-ER 1472 is 0.10 (ref. 7), which is exactly the Libben mean. This specimen is also classified as *Homo*. The only specimen classified as *Australopithecus* to which Wolpoff refers in regards femoral head size and length is STS 14, but this specimen is missing both its head and its distal end⁴. (5) Walker⁵ did not conclude that the "relative head size . . . is above the human mean". He showed that his composite and reconstructed australopithecine femur has a ratio of head size to length slightly below the human mean.

In our view canonical variates analysis is one more useful tool for analysing complex biological shape. The method allows one to take into account variability within taxa, it reduces the complexity of interrelated shapes and sizes to understandable dimensions, and it allows an anatomical region to be treated as a total complex (inasmuch as the available fossil material will allow).

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Agglutinins for complement-coated sheep erythrocytes

SALIVARY 'IgA Immunoconglutinins', which reportedly bind both altered and native C3, have generated considerable interest^{1,2}. Unfortunately, the methods so far described for their purification are somewhat cumbersome, as they involve incubation of complement-coated sensitised sheep cells with saliva and subsequent elution of the 'IgA immunoconglutinins' with a chelating buffer. In our laboratory we have used a more classical approach, first fractionating parotid saliva on BioGel P100, then subfractionating each peak by DEAE-cellulose chromatography and testing the fractions for their reactions with complement and complement-coated cells (Fig. 1). (The details of the purification of salivary complement-reactive factors will be published elsewhere³.)

Our procedure has obvious advantages over that of Price *et al.*¹. Since Ca²⁺-dependent IgA agglutinins for sheep cells are present in low titre in salivary secretions^{3,4}, it is possible that those authors isolated secretory IgA attached not only to the human C3 on

erythrocytes would tend to confuse qualitative and quantitative agglutination results. Furthermore, preliminary evidence suggests that salivary C3

Matters arising

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and C4 agglutinins can be separated by DEAE subfractionation of the BioGel P100 peaks, with some salivary fractions demonstrating agglutinating activity for complement-coated sensitised erythrocytes and yet containing no detectable α -chain antigens.

Using purified saliva fractions we have found that saliva contains agglu-

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PRICE *et al.* REPLY.—The claim by Boackle *et al.*¹ that their purification procedure "has obvious advantages over that of Price *et al.*" is based on the erroneous assumption that calcium-dependent IgA agglutinins to sheep erythrocytes are present in high titre in salivary secretions. In support of this statement they refer to the work of Tönder and Larsen². The IgA agglutinins described by these authors, however, were not calcium dependent and were directed against rabbit rather than sheep erythrocytes. Agglutinins to sheep erythrocytes were looked for and found in only four of 17 samples of saliva, the highest titre being 1:4. Other workers have shown that agglutinins to sensitised sheep erythrocytes are present in low titre in adult saliva, but these agglutinins are not calcium dependent^{3,4}.

Contamination with IgA antibodies directed against the sensitised erythrocytes would therefore not occur when EDTA was used to elute the anti-C3 antibodies from the cell surface. A simple way of preventing possible contamination by calcium-dependent agglutinins would be to incubate the saliva with sensitised erythrocytes before incubation with complement-coated cells.

Other workers who may be interested in studying these antibodies will decide whether EDTA elution from washed complement-coated erythrocytes (a procedure which may be performed in 2-3 h) or fractionating on BioGel P100 and then subfractionating on DEAE-cellulose is the less cumbersome method of purification.

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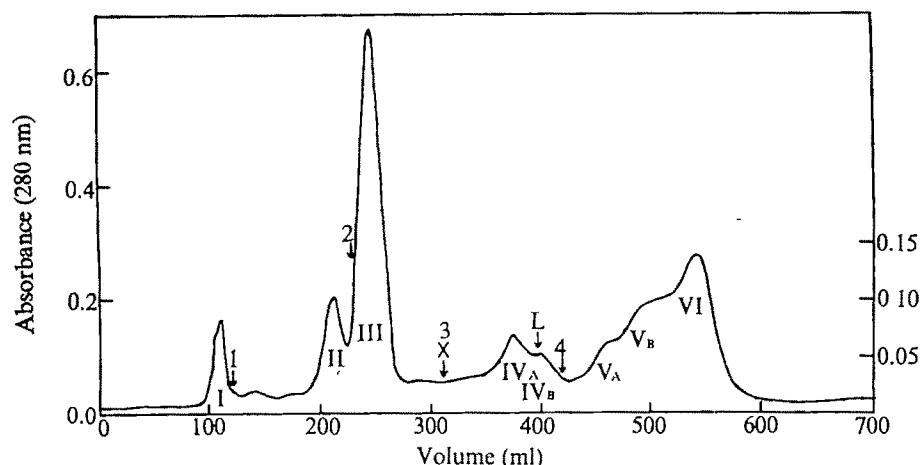


Fig. 1 Molecular sieve elution pattern of pooled parotid saliva chromatographed on BioGel P100. The elution buffer was 0.1 M Tris-0.2 M NaCl, pH 8.1, with a flow rate of approximately 6 ml h⁻¹. Column bed dimensions were 2.5 × 96.5 cm with a volume of 475 ml. The saliva was dialysed against the column buffer using 3,000 m.w. cutoff tubing (Thomas) before concentrating (Amicon apparatus, UMO5). Five millilitres of sample was added to the column. Salivary fractions (SFP) are labelled with Roman numerals. Numbered arrows refer to marker protein elution volumes; the proteins used were bovine albumin, chymotrypsinogen A, insulin, and bovine trypsin inhibitor, for arrows 1-4 respectively. The arrow labelled L represents the elution volume of human lysozyme, which was verified independently, and X denotes the scale change position.

the sensitised erythrocytes but also to the erythrocytes themselves. Contamination with IgA antibodies and non-specific agglutinins for sensitised

tinins for IgG- and IgM-coated erythrocytes, located in SFPIV and SFPII respectively (Fig. 1). α -chain antigens were found only in SFPI

reviews

Geological survey of Greenland

J. Sutton

Geology of Greenland. Edited by Escher and Watt. Pp 603. (Geological Survey of Greenland Østervoldgade 10, DK-1350 Copenhagen K, Denmark, 1976) DKr.195 inc. postage.

COMPARED with many parts of the Solar System, the Earth is a protean creature, in a constant state of change. With such activity and destruction, Earth-bound geologists can never hope to find a landscape carpeted, as on the Moon, with fragments which have lain undisturbed for 3,000 Myr. Yet just occasionally geological circumstances combine to provide an unusually clear view of the geological past. The coastlines of Greenland, scraped bare by an ice sheet which has now drawn back to reveal fresh rock, provide unparalleled opportunities for geological research. This splendid book shows how successfully the Geological Survey of Greenland has grasped these opportunities during its first 30 years of activity.

As Dr Ellitsgaard-Rasmussen, who has directed the Survey for most of this time, points out, the Arctic climate and isolated situation require the mounting of special expeditions to carry out geological research in Greenland. The twenty-one chapters provided by some thirty contributors, most of whom have worked with the Survey, show how ably the staff have mastered logistical problems. With no fuss and great efficiency the Survey moves, feeds and supports its geological parties who are thus able to concentrate on scientific work, and who have surveyed, at least in outline, the entire mountainous seaboard bordering the world's largest island.

The opening sentence of the Director's preface puts the matter with characteristic plainness and brevity: "*Geology of Greenland*", he writes, "aims to provide a concise modern account of nearly all aspects of Greenland's geology". It does precisely this, drawing almost entirely on recent work while recognising the achievements of the pioneers, from the days of Giesecke in the early nineteenth century to the expeditions of Lauge Koch, Wegmann, Watkins and Wager more than a hundred years later.

Every chapter comes at first hand from experts who have been repeatedly to the terrain they describe. Each provides a readable narrative between a dozen and sixty pages in length, excellently illustrated. The figures (there are 470) include a variety of geological sketch maps and photographs that demonstrate the clarity with which geological phenomena are displayed in Greenland. The authors reflect the international nature of the enterprise. The Survey collaborates with more than fifty Universities and Institutes, and moreover does so with a generosity and openness which has attracted able scientists from many countries. The heart of the undertaking lies, however, in Denmark. It is Danish resources and leadership that have brought the Geological Survey of Greenland to its present eminence. Readers of this book have plenty of material on which to assess this judgement.

The opening chapters will appeal to the student of crystalline rocks. Here are described the Archaean and Proterozoic rocks which form the mass of Greenland. The main divisions established in Greenland can be extended into Canada and north-western Europe, and so provide a key to a unified view of the Precambrian of much of the Northern Hemisphere. Of even greater importance, perhaps, is the insight into geological processes of those times, made possible by careful study of excellently displayed terrain. These chapters could influence scientific thought in the way the British Geological Survey's work on Tertiary volcanic centres early this century influenced

the development of petrology. In each instance accounts of newly discovered phenomena prompt inquiries into their origin. One thinks of Bowen's use of British investigations some fifty years ago. An equal opportunity is provided here.

But this is no more than the first course. There follow chapters on the younger rocks of the east, north and west coasts, each of which developed somewhat differently, although all illustrate what may happen before and during the separation of continental blocks. The evolution of eastern Greenland is taken in three excellent chapters from late Precambrian times to the late Tertiary. A similar time span is covered in an account of northern Greenland which provides the first synthesis of that region based on detailed observation. Petroleum prospects, although still at an early stage, are suggested by the accounts of sedimentation around the Greenland coasts, which include short reference to commercial work offshore.

An account of the glaciation and Quaternary history of Greenland completes the geological record of this remarkable island. About fifty pages are devoted to economic minerals, coal and petroleum, followed by an equal number in which plant and vertebrate fossils are described. The book ends with an account of the kimberlites of western Greenland.

J. Sutton is Professor of Geology at Imperial College, University of London, UK.

Essence of Iceland

The Landscapes of Iceland: Types and Regions. By H. Preusser. Pp.xvi+363. (Junk: The Hague, The Netherlands, 1976) DGu120.00.

REGIONAL geography encompasses the systematic patterns of landscape: the physical form and structure, the heat and water available through the seasons and the resulting biotic and human development. Most essays in regional

geography describe the physical characteristics of landscape as a platform or stage on which the life styles of work and play are integrated into a cultural pattern over the environment, increasingly controlled by complex economic forces. If these components are well drawn and emphasis is duly apportioned to each, and differences across regional boundaries are correctly discerned, the essay is then greater than the sum of

its parts and the story told gives the essence, the *entité*, of the region.

This book describes the landscape in great detail and is thorough in defining type areas and the effect of man. The cultural landscape is given less attention because the low population density compels less human emphasis. The book is a doctoral thesis based on an intensive search of the literature (there are 30 pages of bibliography), a detailed interpretation of maps, diagrams, air and satellite photographs and three extensive visits. The author has been fascinated by the sudden changes that the traveller sees in this land of ice and fire, with its awesome interior plateau more like a moonscape than any landscape on Earth.

The first part (100 pages) of the book is a national compendium, defining terms, describing landscape features, mapping climatic elements and listing population and employment changes

and their products up to 1970. The second part of the book (40 pages) describes nine main landscape types. The third part (about half the book) extends this, with 30 geographically located regions, some with further subdivision. It is not easy to know where the author is in these descriptions, although the many diagrams and sketch maps are a great help. It is therefore essential to have the nine Icelandic maps (1:250,000) alongside the book. To capture the spirit of place, the reader should also have some of the many beautifully produced volumes of colour photographs of Iceland.

The book is a scholarly work and is, considering how crammed with information and references, remarkably easy to read

H. Lister

H. Lister is Reader in Physical Geography at the University of Newcastle upon Tyne, UK.

State of the oceans

The Health of the Oceans. By Edward D. Goldberg. Pp. 172. (Unesco Press: Paris, 1976.)

EDWARD GOLDBERG'S book arose as a result of a recommendation made in 1973 at the time of the first meeting of the Intergovernmental Oceanographic Commission's International Co-ordination Group for the Global Investigation of Pollution in the Marine Environment. As originally conceived, it was intended to fulfil the role of a baseline, or statement on the status of pollution in the marine environment, against which to judge the changing health of the oceans within a continuing review of marine pollution as part of the Global Investigation of Pollution in the Marine Environment. A great deal of useful information to that end is contained within the ten chapters, but the book has nevertheless been written against a much broader conception of the problem than was originally intended.

There is an effort to produce a health status report; but also much of a speculative nature written to examine causes, to reflect on philosophy and to make tentative suggestions about future problems and their solutions. The book dwells at length on the lessons to be learned from the successful regulation of radioactive waste disposal to the oceans, on the mercury problem, and on DDT and oil. The author clearly believes that much could be gained by attacking other pollution problems along similar lines to those used in the UK in relation to radio-

activity, including the application of 'critical path' philosophy to the design and implementation of monitoring programmes; a suggestion with which the reviewer has much sympathy.

Halogenated hydrocarbons, radioactivity, heavy metals and oil all receive treatment in separate chapters, supported by chapters which deal collectively with marine pollution dynamics, modelling (especially mass balance studies), monitoring and prediction. Among the problems which obviously concern the author, and which he returns to throughout the text, are the long term significance of small changes in pollutant concentration in the oceans, the difficulty in unequivocally establishing such trends, and our reaction time in taking remedial action when the system's reaction time itself may be long and, as a consequence, re-establishment of normal concentrations may take a long time: a time during which damage to the system may appear which will take longer to rectify and which may have serious consequences as yet unpredictable.

There is a carefully written preface by Alan Holden, and readers of the book would do well to peruse it before tackling the text. One must conclude, however, that Professor Goldberg has picked his way through something of a minefield without much more than superficial wounds, and produced a thought-provoking text on the way.

Alan Preston

Alan Preston is Deputy Director of Fisheries Research in the Ministry of Agriculture, Fisheries and Food based at Lowestoft Laboratories, and Chairman of the UK Marine Pollution Monitoring Management Group.

Updating nematodes

Physiology of Nematodes. Second Edition. By D. L. Lee and H. J. Atkinson. Pp. x+215. (Macmillan London, October, 1976.) £5.95

WHEN Donald L. Lee wrote the first edition of this book it was a milestone. Although of modest size, it was the first attempt to organise the subject, stimulated great interest and highlighted critical areas for the research efforts of others. Now, with his colleague, Howard J. Atkinson, a second edition has been published. In the eleven years between the editions and partly because of the impact of the first, there have been numerous papers, reviews and books on nematode physiology. This new edition still presents an excellent introduction for undergraduates, as it is designed to do, but those professionally involved with nematodes will spot some over-generalisations and omissions.

The chapters include: introduction; cuticle, moulting and growth, feeding and digestive physiology; metabolism (a particularly comprehensive section); osmotic and ionic regulation; excretion, reproductive physiology and hatching, neuromuscular physiology; locomotion; sense organs and behaviour. There are 77 figures, less than 40 of which were in the first edition, and there are 246 references instead of the earlier 161. The references are only representative and no attempt is made to credit individual workers in particular fields. There are few typographical errors but nematode names are occasionally mis-spelt (four in chapter 4) and there are seven errors in the appendix listing nematodes. The text and figures have the same clear, concise and very readable features of the first edition.

Specific points are sometimes unclear: nematodes are still credited with constant cell numbers but this is a much compromised notion now (p27). Predatory nematodes are poorly discussed in the light of the most recent studies (p44). Various reports have shown that the 'lens' of ocelli is, in fact, the photoreceptive rhabdomere and that no lens exists. The figure of *Deontostoma californicum* with a lens is particularly unfortunate as this was the species first shown not to have a 'lens'. Photic response for two species is ignored (p68) which makes incorrect the statements on their behaviour.

At £5.95 this book deserves to enjoy good sales, and it will be widely read.

Neil A. Croll

Neil Croll is Professor of Parasitology and Director of the Institute of Parasitology, McGill University, Montreal, Quebec, Canada.

Liquid and solid helium

The Physics of Liquid and Solid Helium Part 1. (Interscience Monographs and Texts in Physics and Astronomy, Vol. 29.) by K. H. Bennemann and J. B. Ketterson, Pp. 589. (Wiley-Interscience, New York and London, June 1976.) \$35.35, £21.

SOME books are to be read as a whole, others may to greater advantage be dipped into with discretion. This book, consisting of six chapters each of which amounts to a relatively self-contained review article on a specialised topic, clearly belongs to the latter class.

The first chapter, by Khalatnikov on the phenomenological theory of superfluid ^4He has much in common with his frequently cited monograph *An Introduction to the Theory of Superfluidity* (Benjamin, New York, 1965). There follow five detailed and carefully constructed reviews: by Ahlers on experiments near the superfluid transition; by Fetter on ions and vortices; by Stephen on light scattering; by Woo on microscopic calculations; and by Varma and Werthamer on solid helium. Each of these chapters has its own bibliography which, in most cases, is extensive enough to be really useful to anybody working in or wishing to enter the field in question. Fetter, for example, includes more than 500 separate citations.

It is not a book for beginners. Khalatnikov's chapter, although admirable in itself, is too succinct, and too limited in scope, to constitute an adequate introduction to the material which follows. Graduate students will therefore be well advised to read an introductory text, such as the excellent little book by Tilley and Tilley (*Superfluidity and Superconductivity*, van Nostrand Reinhold, London, 1974), before embarking on the sections of the present volume relevant to their research.

Apart from the first, all the topics covered are ones in which there has been considerable progress in recent years. These substantial review articles will thus be of value to research workers in assessing the current position and deciding where further investigations are still required. The absence of a chapter dealing with the rapidly developing field of superfluid ^3He is naturally disappointing, but a certain loss of topicality and impact was inevitable in view of the four years' delay between completion of writing and publication. In most cases the authors have done what they can at the proof stage, through incorporation of

addenda and additional references, to update their original material to 1974 or so. Although the book is clearly a must for libraries, the extent of individual sales is likely, on account of the price, to be somewhat limited. This is rather a pity, because there will undoubtedly be many who would appreciate personal copies of particular chapters, but who will be disinclined, or unable, to pay for all the others which may be of less interest to them.

Part II which, it is promised, will include a couple of chapters on superfluid ^3He will be awaited with eager anticipation. **P. V. E. McClintock**

Dr McClintock is a Lecturer in the Department of Physics at the University of Lancaster, UK.

Flavour of gene expression

Control of Gene Expression. By Norman Maclean. Pp. xi+348. (Academic, London and New York, May 1976.) £7.80, \$19.75.

POSSIBLY because this book arrived on my desk around holiday time I viewed it as a sort of tourist menu attempting to offer a wide array of characteristic foods as an introduction to the culinary arts of a new country. The broad title is perhaps a little misleading for the bulk of the book presents a series of concise introductions to the numerous and diverse systems which have been used to study particular aspects of gene expression in eukaryotes. These systems are catalogued under three headings: systems involving control of a single type of protein, systems described as being of limited complexity; and systems classified as "not well understood in molecular terms". These three chapters constitute the meat of the book, the merits of which must rest on the strength of these three chapters.

In describing the systems involving one type of protein the author has clearly set himself a difficult task. The result is an acceptable précis, brief enough to be read by the enthusiastic undergraduate; barely detailed enough for that undergraduate's teacher, but dotted with key references for further reading. Moreover the author has commented in each case on some of the uses to which each system has been, or might be, put.

There is obviously a continuum between systems of limited complexity and systems described as "not well understood in molecular terms". The point at which the author draws the line between these two categories must be arbitrary. It is in these two chapters

that I think the book will come into its own as an introductory text for biochemistry and molecular biology undergraduates. The wide range of fascinating systems are dealt with briefly but interestingly. If any of these accounts serves to lead students trained in the molecular sciences into the study of one of these fascinating systems then the author will be able to congratulate himself.

The book leads off with a chapter on control of gene expression and its level of action, followed by a brief account of gene expression in prokaryotes. At the end there are chapters on RNA involvement in gene expression and general concepts of gene regulation. Each of these chapters is well laid out and might be taken as a separate reading, although read together some parts are a little repetitious.

As a tourist menu of the area of gene expression in eukaryotes the book succeeds in giving a taste of many systems, some fashionable and some less so, and in a number of instances it manages to convey authentic flavour.

A. R. Williamson

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Environmental Chemistry. By John W. Moore and Elizabeth A. Moore. Pp. xv+500. (Academic, New York and London, April 1976.) \$27.50, £15 10.

MANY environmental problems have been initiated by the failure of decision-makers to consider problems in their entirety and the failure of specialists to make relevant information both available and understandable. American science students having pursued the popular, mind-broadening course on which this book is based will be better able to fulfil their roles in both science and society irrespective of the careers they choose. Most suited to the interests of chemists having acquired a basic knowledge of kinetics and thermodynamics, the book should be considered essential reading for degree level courses in chemistry or environmental science. Physicochemical principles are applied to a breadth of practical non-ideal systems with economic, historical, and social commentary in an enjoyable and readable manner.

Following an introductory section on chemical evolution, the main text is based squarely on the alchemical elementary concepts of fire (energy), air, earth and water illustrated using modern issues. Nuclear reactor safety, stratospheric ozone depletion, phosphate eutrophication and use of pesticides are some of the controversial subjects discussed in a balanced way from an American viewpoint. Topicality will date the book rapidly, however, as evidenced by the now rejected Ca-Hg-Br system quoted to explain the hydrogen economy. Exercises and further reading suggestions are included in each chapter. The author's style, free from American phraseology, will appeal to students but many will consider the Moores' first textbook rather expensive.

I. R. McKinley

Examination and Analysis of Starch and Starch Products. Edited by J. A. Radley. p. vii+220. (Applied Science, London, 1976.) £15.00.

This is a practical book giving a description of various starches and the methods for identifying and characterising them.

G. E. Moss describes methods for examining starches in the light microscope. The appearance of various starches is given with numerous illustrative photographs. A table provides information on the microscopic appearance of 32 different starches. D. J. Gallant and C. Sterling describe

both the transmission and scanning electron microscopes and their application to starches. A. H. deVilligen discusses the rheology of different starches. A chapter by the editor follows, in which rheological measurements are discussed along with a description of various commercial viscometers. Procedures are also discussed for measuring gels, colour and several other properties. F. A. Lyne describes methods for determining moisture, mineral matter, fats, protein, alkali number, amylose content, carboxyl groups and so on and also provides procedures for the

Books brief

determination of starch in various products. J. van der Bij provides methods for the analyses of starch ethers and esters.

Each chapter has many references to the fundamental literature. The book should be useful in analytical laboratories concerned with starch analysis. Although detailed methods are not often given, sufficient references are present to lead to the appropriate details where needed.

Roy L. Whistler

The World Computer Chess Championship. Stockholm, 1974. By Jean E. Hayes and David N. L. Levy. Pp. v+105 (Edinburgh University: Edinburgh, May 1976.) £3 75.

THE first World Computer Chess Championship took place in Stockholm in August 1974, and resulted in a closely contested victory for the Soviet Union's 'Kaissa' program, ahead of several American rivals. This book, written by a professional chess master and a research worker in artificial intelligence, combines a report of the tournament games with a brief introduction to the problems of programming a complex human skill into a computer.

Although the account presents the intended material in a well organised manner, I felt that it has fallen short of what might have been achieved. The account of the games is rather pedestrian, following too closely the style of annotation appropriate to human chess, and laying insufficient

emphasis on the peculiar motives and aberrations of computer play. Furthermore, the delay of nearly two years between the tournament and the appearance of the book is far too long in such a rapidly developing subject, and this, coupled with the use of the obsolescent English Descriptive notation (which has all but disappeared from international chess) produces the impression of a historical document rather than an up-to-date report.

Compared with the much earlier report on the same tournament by Monroe Newborn, I find the present book difficult to recommend. It does however, have the merit of being considerably cheaper than the earlier report.

Nigel J. Holloway

Flora Europaea Vol. 4: Plantaginaceae to Compositae (and Rubiaceae). By T. G. Tutin *et al.* Pp. xxix+505+5 maps. (Cambridge University: Cambridge: London and New York, August 1976.) £25.

THE production of *Flora Europaea* is a remarkable project in a number of respects. The geographical area and habitat diversity of the European continent makes it rich in plant species, so the description of its flora is a formidable task. It calls for a highly concise but informative text and in this respect *Flora Europaea* is eminently successful. It is also a credit to authors, editors and publishers in that the appearance of new volumes is a regular and frequent event.

Volume four is now in the bookshops and it maintains the standards of brevity set by its predecessors. The bulk of this volume is taken up with the family Compositae, which brings with it the taxonomic challenge of such genera as *Taraxacum* and *Hieracium* with their multitude of microspecies. The authors deal with these groups in a pleasantly conservative manner. Those of us who are consumers rather than producers of things taxonomic will welcome this agglomerative rather than divisive approach. There is one point, however, where a little splitting might have been welcome, that is in the case of *Hieracium pilosella*. There is a case, one would have thought, for its removal from this large genus.

As a source of concise information, relevant to the needs of taxonomists, ecologists and biogeographers this series is exemplary. The price is not unreasonable by modern standards, although subscribers may feel trapped in the general price escalation of the series.

Peter D. Moore

obituary

Lars Onsager, who died on October 5, 1976, was undoubtedly one of the great scientists of the 20th century. His publications were neither lengthy nor numerous. When he was awarded the Nobel prize in Chemistry for 1968 for his discovery of "Reciprocal relations in irreversible processes", the citation noted that the publication (two papers in *Physical Review*¹ containing 22 and 15 pages respectively) was one of the smallest ever to be awarded a Nobel prize.

Onsager's total publication list runs to some 60 papers, a number which can readily be matched by most university professors of science. But the quality of his publications is superb, and he did not put his name to anything trivial or unfinished. Besides non-equilibrium thermodynamics, a field initiated by his Nobel prize work, there are at least five fields his contributions to which ensure him a place of distinction. In the area of phase transitions many regard his exact solution of the two-dimensional Ising model as of comparable significance to his Nobel prize work; and in the theory of electrolytes, liquid helium, metals and ice, many of his ideas have by now been incorporated into standard texts. He also devoted thought and attention to the problem of turbulence in fluids. He provided a magnificent counter-example to the "publish or perish" philosophy which has influenced so many scientists during the past few decades; and there was a general consensus of opinion among those who had direct contact with him that the unpublished contents of his drawers would have sufficed to establish the reputations of quite a few scientists with less exacting standards than his own.

Despite the clarity of his thinking and writing Onsager was a poor lecturer, and usually pitched his exposition far above the heads of the audience. This was not due to any conscious desire to show off (which was quite alien to his nature), but to a complete lack of appreciation of the gap between himself and his listeners. An incident which occurred during his sabbatical leave at Cambridge in 1951–1952 illustrates this well. Onsager was asked to talk to the Kapitza Club about his work with Bruria Kaufman on the Ising model. The Kapitza Club (named after its founder) aims to provide a meeting ground between theoretical and experimental physicists. In



Photograph taken by Z. R. Hasan, a graduate student of Temple University, during a visit by Onsager a few months before his death.

his briefing Onsager was warned that since there would be experimentalists present he should phrase his talk in a language which would be intelligible to them. Nevertheless, he started with the mathematics of spinors. After a few minutes the thought occurred to him that some of the audience might not have come across spinors before, and he turned to ask if there was anyone present who did not know what a spinor was. One or two brave spirits raised their hands, and Onsager said "Right, I will tell you; spinors are matrices isomorphic with the rotation group in three dimensions" and he continued his talk assuming that this was a sufficient explanation for an experimental physicist!

During the same year he gave a seminar at Oxford about his ideas on the theory of liquid helium, and on this occasion even the theorists were at a loss in trying to follow his arguments. Onsager's final comment in reply to a discussion question was "The results are not bad when you consider the enormity of the swindle which I have perpetrated". It was only a few years later when Feynman's theory of liquid helium appeared with acknowledgements to Onsager's ideas that light was shed retrospectively on what Onsager had been trying to convey in the seminar.

In spontaneous discussion at conferences he came over better, and was

often entertaining as well as informative. Onsager would get up at the end of a long and sophisticated lecture and say "I think Dr X has tackled this problem in a very effective manner, and his first approximation is in the right direction; if he proceeds to a second approximation he should find the following . . ." And he had an endearing habit of going to the front of a distinguished audience, and turning to them with a beaming smile to ask practical down-to-earth questions like a schoolteacher. "Does anyone know what is the best substance to use to illustrate A, B, C? Well, it is sulphur, because . . ."

His knowledge of different aspects of science was immense. When taken round a laboratory he would comment pertinently on every detail of the experimental set-up, to the great surprise of the experimentalists who knew of him only as an abstruse theoretician. And he took it for granted that every research scientist would be familiar with all text-book material—"But it's in all the standard books on palaeontology".

In private discussion it was much easier to communicate with Onsager provided that you were courageous enough to persist in questioning when you did not understand. He would drop the level one stage at a time until the gap could be bridged. Thus he had many disciples and collaborators who uniformly testify to his generosity, and to the inspiration of working with him on scientific problems.

Onsager was born in Norway in 1903. He entered the Norges Tekniske Høgskole in Trondheim in 1920; his first research ideas developed here and were concerned with the theory of electrolytes. After graduating in 1925 he went to the Eidgenössische Technische Hochschule in Zurich to work with Debye and Hückel, and his first publications² were devoted to a revision of the Debye-Hückel theory.

In fact, it was the combination of diffusion and electrical conduction in electrolytes for which he found that reciprocal relations were satisfied which led Onsager to a deeper study of their origin. Although the second law of thermodynamics gives precise information when applied to equilibrium phenomena and reversible processes, for irreversible processes it provides only the qualitative information that the entropy increases. Kelvin had attempted to extend thermodynamics

beyond the equilibrium state in his theory of thermoelectricity, and Helmholtz had derived a "Principle of least dissipation" which Onsager recognised as being equivalent to the reciprocal relations. Onsager had also been thinking about the condition of detailed balancing used widely in the theory of chemical reactions and usually taken to be a consequence of the second law. He became convinced that there was nothing in the second law to prevent equilibrium being maintained by cyclic processes when there were more than two independent reactions. A new hypothesis of microscopic reversibility was needed to exclude this possibility.

In his Nobel prize papers¹ published in 1931 Onsager tied all these ideas together, and showed that the reciprocal relations and principle of least dissipation could be derived from this hypothesis. He thereby established a new branch of thermodynamics. For some years little attention was paid to his papers; but in the post-war era the subject of irreversible thermodynamics gained momentum steadily and numerous applications arose in physics, chemistry and biology as well as in technology.

In 1928 Onsager had emigrated to the USA. He spent one term at Johns Hopkins University and five years at Brown University. In 1933 he went to Yale where he stayed until his retirement in 1972; from 1945-72 he served as Josiah Willard Gibbs Professor of Theoretical Chemistry.

His classic paper giving the solution of the two-dimensional Ising model² was published in 1944. The paper was a mathematical *tour de force* and amongst other skills showed remarkable dexterity in handling elliptic and elliptic modular functions. (Onsager once confided to Elliott Montroll that whilst a student he had worked through every example in Whittaker and Watson's *Modern Analysis*!) For the first time the exact statistical mechanics of a realistic model of interacting systems became available. Ideas about the nature of critical points and the behaviour of thermodynamic functions in their neighbourhood had to be completely revised; the publication laid the foundation of the modern era of phase transitions.

The 1944 paper dealt with thermodynamic functions in equilibrium. Of no less importance are the correlations between spins at different distances, and in collaboration with Bruria Kaufman³ Onsager published a paper in 1949 devoted to their behaviour. In the same year he announced cryptically at a conference in Florence⁴ that he and B. Kaufman had also solved the problem of long-range order for a rectangular net and that it was simply $(1-k^2)^{1/8}$ (where k is a simple function of the

interactions). This was a result of the greatest importance, which was re-derived independently by Yang⁵ in 1952. Onsager never published any details of his derivation, and gave no clue to the mathematics he had used. It was only twenty years later at a conference in Gstaad to celebrate the 25th anniversary of the publication of his 1944 paper that some information was forthcoming⁷. In computing the long-range order he had been led to a general consideration of Toeplitz matrices, but he did not know how "to fill out the holes in the mathematics, the epsilons and deltas", and by the time he had achieved this satisfactorily he found that "the mathematicians got there first".

There are many jewels to be found among Onsager's publications and those of others which incorporate his ideas; like the duality relation in two-dimensional Ising nets⁶, the proof of the existence of a Bose-Einstein condensation in interacting systems⁸ (with Penrose), and a novel method of looking at the de Haas van Alphen effect¹⁰. He returned several times to the theory of electrolytes where his researches started (particularly in collaboration with Fuoss).

After retiring from Yale in 1972 Onsager went to the University of Miami. He remained healthy and vigorous until his death; the photograph was taken only a few months before he died.

Onsager held honorary doctorates of several universities, including Cambridge and Oxford. Among his many awards were the Rumford Medal of the American Academy of Arts and Sciences, the Lorentz Medal of the Royal Netherlands Academy of Sciences, and the National Science Medal. He was elected a Foreign Member of the Royal Society in 1975. His colourful personality will be missed particularly at international gatherings.

C. Domb

¹ Onsager, L., *Phys. Rev.*, **37**, 405-26; **38**, 2265-79 (1931).

² Onsager, L., *Z. Phys.*, **27**, 388-92 (1926); **28**, 277-98 (1927).

³ Onsager, L., *Phys. Rev.*, **65**, 117-49 (1944).

⁴ Kaufman, B., and Onsager, L., *Phys. Rev.*, **76**, 1244-52 (1949).

⁵ Discussion remark in *Nuovo Cim*, Ser. IX, **6**, (Suppl.) 261 (1949).

⁶ Yang, C. N., *Phys. Rev.*, **85**, 808-16 (1952).

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Dr William Nordberg, internationally known space scientist and Director of Applications at NASA's Goddard Space Flight Centre, near Washington, D.C., died on October 3, after a two year illness, from cancer. Born in 1930, Dr

Nordberg was educated at the University of Graz in Austria, his native country. He emigrated to the U.S.A. in 1953 and after working for the U.S. Army Signal Corps as an atmospheric physicist, particularly on the International Geophysical Year rocket programme, he joined the newly formed NASA at the Goddard Space Flight Centre in 1959. His first post there was head of the physical measurement section, Meteorology Branch in the Satellite Applications Division. From there he progressively rose to higher management positions until his appointment as Director of Applications in 1974.

From the beginning of the U.S. satellite programme Dr Nordberg applied his enormous enthusiasm and powers of leadership to pioneering the development of remote sensors for observing the atmosphere from space vehicles. He was involved in the instrumentation of the early Tiros weather satellites and many of the infra-red radiometric instruments on the first Nimbus meteorological research satellite were developed and built under his direction. He was quick to realise the potential of remote sounding measurements, not only for research on the atmosphere, but also for the investigation of the properties of the earth's surface, and under his leadership microwave instrumentation for mapping the distribution of sea ice and other important surface features was flown. The success of the Landsat satellites, which have carried remote sensors for mapping a wide range of earth resources, owed not a little to Dr Nordberg's foresight and ability to organise and to interest people from a wide range of scientific disciplines. He successfully co-ordinated the activities of 300 principal investigators from 38 countries in the use of Landsat data.

Dr Nordberg received a number of awards including NASA's highest award, the Distinguished Service Medal. In September of this year, shortly before his death, he was elected to be a Fellow of the American Meteorological Society, a fitting tribute to his pioneering work in space meteorology.

Bill Nordberg, through his extensive travels, made friends in almost every country of the world. He will always be remembered for his enormous energy, his infectious enthusiasm for science and his tremendous zest for life which continued even right through his long and trying illness. By his tragically early death the international space research community has lost an outstanding scientist and many of us have lost a true and valued friend.

He leaves his wife and his parents and brother in Austria.

J. T. Houghton

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